Egg production, flight velocity and predation risk in birds

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Contents

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Abstract

The costs of reproduction can be defined as the trade-off between present and future reproduction, where current reproduction may diminish future reproductive success of the parent and/or of the parent's offspring. One potential cost of reproduction in birds may be a reduction in the ability of the female to escape from predators due to a reduction in maximal flight velocity. Such a reduction in flight performance may come about in laying females as a result of an increase in mass over the laying period, and a reduction in flight muscle condition. The result of such a reduction in the flight velocity of wild birds would be to increase the susceptibility of those birds to capture by predators once attacked. To investigate the potential of egg production to affect flight velocity and consequently predation risk, the individual effects of body mass and muscle condition needed to be determined.

Contrary to theoretical studies which have indicated that body mass might significantly affect flight velocity in birds, no such relationship was found in startled zebra finches (*Taeniopygia guttata*), a finding supported by a number of other empirical studies on other species. To determine whether muscle loss is significant in affecting flight velocity, the physiological costs of reproduction and consequently the extent to which females lost muscle condition were manipulated. This was achieved by encouraging females to lay large or small clutches on high or low quality protein diets, and measuring the changes in muscle condition and flight velocity during the course of laying a clutch. A strong positive relationship was found to exist between changes in muscle condition and changes in flight velocity. Treatments in which birds exhibited the largest declines in muscle condition, were associated with the largest declines in flight velocity. In contrast to this, those treatments in which birds lost the least muscle condition were associated with actual improvements in flight performance, that is to say, birds flew faster at the end of a clutch than at the beginning. These changes were independent of body mass and occurred post-laying indicating that it is the cost of egg production that is critical in affecting flight velocity rather than the cost of carrying eggs as had previously

been suggested. Moreover, it is demonstrated that independently of diet and changes in body mass, increased reproductive output decreases maximal flight velocity mediated by an increase in muscle loss. This is the first demonstration of a physiological trade-off between resource allocation to reproduction and the maintenance of musculature critical to an animal's escape response. Reduced muscle condition as a result of laying might thus constrain clutches sizes in birds vulnerable to predation. Evidence was then found that females may store proteins within their muscle tissue for egg production, since females had greater flight muscle to body mass ratios than males. This proved to be costly to females since flight velocity negatively correlated with muscle condition index (an estimate of muscle volume), whereas no such relationship existed in male birds. This confirms that the costs of reproduction can occur at all stages of the reproductive cycle.

The costs of egg production in terms of reduced flight velocity post-laying is shown to be state dependent: females laying clutches having been given a high quality protein pre-breeding diet, demonstrated improvements in flight velocity, whilst when having laid the same sized clutch on a low quality protein pre-breeding diet, declines were seen in their flight velocity. This difference was brought about by the differential shrinkage of the pectoral muscles of the laying birds, and was independent of body mass, clutch size or clutch mass. This is the first demonstration of diet quality affecting predator evasion and illustrates state dependent costs of reproduction, independent of reproductive output.

Since a trade-off between egg production and predation risk has been demonstrated for females, one might expect females to alter their behaviour in response to this change. Female zebra finches were shown to take fewer risks in the week following laying, than in the two weeks which preceded laying. These effects were enhanced if females were deprived of cover. Consequently, female zebra finches appear sensitive to changes in their predation risk, and able to adapt to these changes. The findings so far described clearly demonstrate that egg production may well be costly in birds. This has

implications upon the practice of egg-pulling; a conservation tool where eggs are removed from the nests of wild birds to increase fecundity in endangered species. The potential costs of egg-pulling which are considered include reduced female condition, reduced offspring quality and an imbalance of offspring sex ratio. It is suggested that much of these costs can be minimised by the provisioning of appropriate amino acid supplements, thus enhancing the conservation value of this technique.

Chapter 1

General Introduction

On a daily basis, animals may suffer numerous set-backs such as a reduced food intake or failure to obtain matings, but these may have only a minor effect upon the animal's lifetime fitness. In comparison to this, the failure to avoid predation will have a far more significant fitness cost. Consequently, for many species, predation pressure is a very powerful selective force, and the arms race between predator and prey is such that slight changes in the ability of a prey species to evade a predator will have disproportionate effects upon their survival, and subsequently their fitness. This is particularly so of small ground feeding birds who are exposed to predation when feeding and are reliant upon speed of flight to escape pursuing predators (Rudebeck 1950, Page & Whitacre 1975, Cresswell 1993) to such an extent that it has been suggested that predation risk per attack will increase exponentially with time taken to reach cover (Bednekoff 1996). Subsequently even small reductions in flight velocity may profoundly effect a bird's chances of survival when under attack from a predator.

In comparison to the short term seasonal or daily fluctuations in predation risk, over evolutionary time predation pressure may be relatively invariant (Lima & Dill 1989). Predation risk may for example, vary on a seasonal basis, such as is the case for migrant turnstones (*Arenaria interpres*) who are subject to sparrowhawk (*Accipiter nisus*) predation on arrival at their wintering grounds in south-east Scotland (Whitfield 1985), or even on a minute to minute basis, such as when willow tits (*Parus montanus*) leave cover to feed and consequently expose themselves to predation (Hogstad 1988).

Reproduction in birds may represent another potential short term factor which may significantly effect their risk from predation. Evidence exists that in a number of animal groups the condition of an individual may decline during the reproductive period in such a way as might impair their chances of survival, if

not their risk from predation. In red deer (*Cervus elaphus*) for example, adult mortality is higher for nursing females than non-nursing females due to an inability to lay down fat until weaning has occurred (Clutton-Brock *et al.* 1989), and in the collared fly catcher (*Ficedula albicollis*), enlarged brood sizes result in increased parasitic infections (Gustafsson *et al.* 1994), and a reduced immune response (Nordling *et al.* 1998). Some studies however, have shown that the ability of an animal to evade predation may also decline as a result of reproduction. Slagsvold & Dale (1996) have shown that the predation rate of pied flycatchers (*Ficedula hypoleuca*) is at its highest during the egg laying phase, whilst Madsen (1987) has shown that the grass snake (*Natrix natrix*) suffers from decreased locomotor capabilities when gravid and is subsequently more vulnerable to predation.

It is possible that, as in the case of the collared flycatcher, predation rates may increase in numerous bird species during the egg laying period. There is however, a lack of evidence to support this, for a number of possible reasons: 1. Research has not paid adequate attention to the effects of egg production upon predation risk in birds. 2. Wild populations of predatory birds have been reduced over the last century, and subsequently predation rates may have been artificially depressed, thus leading to an underestimation of the importance of predation on many bird species (Lima 1987a). 3. Studies investigating the reproductive biology of birds tend to reduce predation rates due to the presence of humans and the use of predator proof nest-boxes (Stearns 1992). 4. It may be impossible to attribute the disappearance of a bird to a predation incident, and so estimates of predation rates, are at best, only that.

Even if increased predation risk is a cost of reproduction in birds, there are even less data concerning the mechanisms which may underlie any such increases. Numerous aspects of the reproductive period may expose individuals across a wide range of taxonomic groups to an increased risk of predation. Nest building, for example, has been shown to elevate predation rates in pied flycatchers (Slagsvold & Dale 1996), and attempts to attract a mate made by the Túnara frog (*Physalaemus pustulosus*), also serves to attract a number of

predatory animals (Ryan 1985), whilst amplexus in the amphipod *Gammarus* (*spp*) reduces the mobility of the mating unit and in doing so makes them more vulnerable to predation (Ward 1986). It is possible that in birds as in some reptiles (Schwarzkopf & Shine 1992), egg production may have an effect upon predation risk through its effects upon maximal escape velocity.

Egg production may potentially result in reduced maximal flight velocity and subsequently increased predation risk in female birds for two main reasons. The first reason concerns changes which occur in maternal muscle condition during laying, whilst the second concerns changes in maternal body mass.

It has been shown that the loss of flight muscle condition during the course of egg production is a widespread phenomenon amongst birds. Twenty one out of 29 bird species studied exhibit such declines in flight muscle condition during laying (Houston *et al.* 1995a). These losses may be substantial. Kendall *et al.* (1973) for example found that losses in muscle weight of red-billed quelea (*Quelea quelea*) during laying represent approximately half the difference between peak and starvation conditions, whilst Ankney (1984) found that breast muscle condition in the brant goose (*Branta bernicla*) declined by 46.3% over the laying period and into the start of the moult. It has since been shown that in the zebra finch (*Taeniopygia guttata*) these losses in muscle tissue represent a direct contribution of muscle proteins to the formation of eggs (Houston *et al.* 1995b). Moreover, it has been shown that flight muscle ratio (i.e. the ratio of flight muscle mass to body mass) significantly effects take-off ability, with animals with lower flight muscle ratios taking off at slower speeds and shallower angles (Hartman 1961, Marden 1987).

In addition to this, it has been shown that during the egg laying period birds may be between 7-50% heavier than when in non-breeding condition depending upon the species (Moreno 1989, Perrins & Birkhead 1983). This increase in mass is a result of the weight of the developing eggs retained in reproductive tract and the gonadal hypertrophy that occurs at this time. Theoretical studies carried out by Pennycuick (1969), Rayner (1979) and

Ellington (1984) suggest that such increases in body mass will impair take-off performance since they claim that maximal lift production is inversely proportional to the square root of wing loading, for which there is some supporting empirical evidence. Jones (1987) found that female sand martins (*Riparia riparia*) were slower to gain height during the pre-laying period, and Metcalfe & Ure (1995) found that the 7% daily increase in mass observed in zebra finches was associated with a 20% reduction in vertical flight velocity, and a 32% increase in time taken to complete a vertical slalom course. In addition to the increase in body mass, the position of the centre of body mass will shift with egg load. Movement of the centre of body mass away from the wing base will reduce speed and manoeuvrability (Lee *et al.* 1996). The combination of an increase in body mass with a decrease in flight muscle size and condition will significantly reduce the flight muscle ratio, and is thus likely to reduce the take-off performance of the female (Hartman 1961, Marden 1987). This is likely to be particularly pronounced at the end of laying, since at this stage flight muscle depletion is likely to be at its maximum, and the reproductive organs will not yet have fully regressed (Moreno 1989).

The aim of this study was to investigate whether the avian reproductive cycle affected flight performance (i.e. velocity), and to tease apart the effects of changes in body mass from the changes in flight muscle condition. Chapter 2 deals with establishing the nature of the relationship between body mass and flight velocity that is relevant to predation risk. The work of Metcalfe & Ure (1995) concerned the relationship between body mass and routine flight velocity. However, this cannot be assumed to be applicable to the type of flights made when escaping predators. This work was repeated therefore, but investigating the effect of mass upon maximal alarmed flight velocity. To do this females were encouraged to make alarmed flights from take-off at various times of the day. In doing so, the diurnal variation in body mass that is known to occur in the zebra finch was utilised (Dall & Witter 1994), which allowed flights to be made over a wide variation in body mass for the same individual. Chapter 3 aims to establish the nature of the relationship between changes in muscle condition with changes in maximal flight velocity during laying. To do

this, changes in muscle loss are manipulated by experimentally varying the clutch sizes laid (see Haywood 1993ab) and also the pre-breeding diet quality of the females. Changes in flight velocity over the course of a clutch are then regressed against changes in muscle condition index, with corrections being made for the changes in body mass. Chapter 4 aims to investigate whether there is any physical evidence that females store proteins for egg production, as had been suggested by Kendall *et al.* (1973) and Houston *et al.* (1995bc) and whether this is costly to them in terms of flight velocity. To do this, the relative size of pectoral muscle tissue in males and females are compared, and the relationship between muscle size and flight velocity in non-breeding males and breeding females investigated. Chapter 5 aims to investigate the effects of reproductive output upon predation related costs in the zebra finch, particularly since it is predicted that increased reproductive output will yield increased fitness costs (Stearns 1992), and in lesser black-backed gulls (*Larus fuscus*) the number of eggs laid has been shown to influence changes in flight muscle condition (Monaghan *et al.* 1998) which may in turn affect flight performance. In order to do this, females are manipulated into laying large and small clutches, and the changes in flight performance are compared between the two treatments. In Chapter 6 the effect of maternal condition (i.e. state) upon predation related costs of reproduction are investigated since changes in muscle condition have been shown to be dependent upon pre-breeding diet quality (Selman & Houston 1996a), and evidence is accumulating that decisions concerning reproductive output may be state dependent (McNamara & Houston 1996), implying that reproductive costs may also be state dependent. In order to do this, females are encouraged to lay similar sized clutches under both a low and a high quality dietary regime, and the changes in flight performance at the end of the clutch are compared across the two treatments. Chapter 7 investigates the extent to which females are capable of adjusting their behaviour to minimise their risk from predation, and the extent to which this is compromised by changes in the female's relationship with the male. Since animals must do many things that may compromise their ability to avoid predators, one would expect animals to be sensitive to current levels of predation and be able to adapt to them. If reproduction does indeed result in an

increase in predation risk due to a reduction in flight performance, one would expect birds to modify their behaviour to mitigate this. Such behavioural changes are most likely to be evident during feeding since it is at this time that ground feeding birds will be most susceptible to predators due to an inability to maintain constant vigilance, and because they are often forced to feed in exposed locations. In order to examine this, females were allowed to breed in large outdoor aviaries where they could adjust their exposure to risk by choosing to feed in high or low risk food patches, by varying the extent to which they flocked with other birds including their partner and the extent to which they left cover.

Although the research contained in this PhD is not of an applied nature, it may have useful conservation applications. In Chapter 8 the relevance of these findings to the practice of clutch manipulations in endangered wild birds is discussed.

The same method of data collection was used in chapters 2-6, with flight times recorded over 15 cm, 30 cm and 115 cm distances. In all of these chapters, only the most significant results are discussed, in no cases were results for one height contradictory with those of another unless otherwise stated. In all, eight breeding trials were carried out. Data from these are used in the analyses of chapters 2-6.

Chapter 2

A reassessment of the effect of body mass upon flight speed and predation risk in birds

Abstract

A number of theoretical studies have predicted that avian predation risk is mass dependent. Models of bird flight predict that increased mass will reduce flight velocity, making birds more vulnerable to predators. Empirical studies supporting this have demonstrated a significant relationship between mass and routine (i.e. unalarmed) flight velocity. Studies investigating the effects of mass upon flight velocity when birds are alarmed however, tend to show that the mass/velocity relationship is less marked. We demonstrate a real difference in the relationship between mass and flight velocity in zebra finches (*Taeniopygia guttata*) when comparing routine and alarmed flights. Despite the strong mass/routine velocity relationship already demonstrated for zebra finches, mass tends to be a poor predictor of alarmed flight velocity within the natural weight range of the species. This difference appears to be partly due to alarmed birds increasing their velocity more when heavy than when light, in comparison to their respective predicted routine flight velocities for their weights. As a result, mass/velocity regression slopes tend to be more shallow for alarmed than for routine flights. Consequently, increases in body mass within the natural weight range of a bird, may have less effect on alarmed flight velocities than they do on routine flights velocities. We therefore recommend caution in the use of weight as a predictor of predation risk in birds without examining its effects upon alarmed flight velocity. A number of possible explanations for the differences in the mass/velocity relationship between routine and alarmed flights are suggested.

Introduction

In avian prey species, predation must be considered as one of the most powerful selective forces in the evolution of behavioural and morphological characteristics. It has been suggested that predation risk in birds is mass dependent, through its effect on flight performance. Certain aspects of flight performance, especially parameters that increase the time taken to reach cover, such as take-off speed, flight velocity, and manoeuvrability, are vital in the escape response repertoire of prey species, in particular for small ground feeders. Attacks on small birds are most effective if the prey can be surprised and caught whilst still on the ground, as once airborne a small bird's chances of being caught are much lower (Temeles 1985). Predators generally attain speed through diving. Prey species in turn attempt to out-climb pursuing predators as the chances of capture are much reduced if they can stay above the predator (Howland 1974). Cresswell (1993), for example, found that redshanks (*Tringa totanus*) suffered an 8% capture rate if they were in flight when attacked by sparrowhawks (*Accipiter nisus*), compared with a 91% capture rate if they remained on the ground. In addition, many species of small birds form flocks when feeding and benefit from the dilution, confusion and selfish herd effects as reviewed by Grier & Burk (1992). Such co-ordinated behaviour makes it essential for individuals to be able to take-off and manoeuvre rapidly, as stragglers will be at a greater risk of predation from both aerial and ground dwelling predators (Hamilton 1971; Davis 1975; Kenward 1978).

Theoretical work on animal flight has for a long time suggested a link between body mass and take-off performance. Hartman (1961) suggested that take-off ability was determined by the flight muscle ratio (the ratio of flight muscle mass to total body mass). Pennycuick (1969) suggested that maximum lift production was inversely proportional to the square root of wing disk loading. There is also indirect empirical evidence of mass having an effect upon avian predation risk, such as a reduction in mass or fat storage in relation to an increase in perceived predation risk (Grant 1965; Blem 1976; Gosler *et al.* 1995; Lilliendahl 1997). Direct studies on the effects of mass upon flight

performance have, however, been somewhat less consistent in their findings. A number of studies have demonstrated that the aspects of flight critical to escape from predators may be impaired by an increase in body mass (e.g. Marden 1987; Hedenström 1992; Witter & Cuthill 1993; Metcalfe & Ure 1995). Metcalfe & Ure (1995), for example, found that a mean increase of 7% in the mass of zebra finches during the course of a day was associated with a 20% reduction in vertical flight velocity, and a 32% increase in time taken to complete a vertical slalom course. Studies on climbing rates of different species of bird have demonstrated that heavier species tend to have slower climb rates (Hedenström & Alerstam 1992). These, and other similar findings, suggest a potential and yet substantial fitness cost for prey species as a result of being heavier.

However, the majority of studies on mass/flight performance relationships have been carried out using routine, that is to say unalarmed, flights. Studies that have used alarmed flights have found somewhat less convincing relationships between mass and flight velocity. Metcalfe & Ure (1995) found that mass had a smaller effect upon flight velocity when birds were alarmed, although a significant mass/alarmed velocity relationship was demonstrated for data pooled from a number of birds. Kullberg *et al.* (1996) using data pooled for a number of birds, found that although increased fat loads did affect velocity in black caps, (*Sylvia atricapilla*), it did so to a much lesser extent than one might have expected from previous studies on routine flights. Witter *et al.* (1994) found the addition of weights to starlings, (*Sturnus vulgaris*), resulted in a lower angle of ascent, but did not significantly affect alarmed flight velocity, implying a possible trade-off between velocity and angle of ascent.

The apparent disparity between the results obtained for alarmed and routine flights means that experiments investigating routine flights alone may potentially over-estimate the increase in predation risk as a result of elevated body mass. In order to establish the true effect of mass upon escape velocity, it is necessary to study the flight of birds under conditions as comparable as possible to the type of flights made by birds under threat from predation. In this

study, therefore, we compared the mass/velocity relationships of routine and alarmed flights, using new data on vertical alarmed flights together with vertical routine and alarmed flight data derived from Metcalfe & Ure (1995).

Methods

Data on alarmed flight performance were obtained from 10 non-breeding female and 12 male zebra finches, from the same genetic stock as those used by Metcalfe & Ure (1995). This species was chosen to ensure that comparisons could be made with the data from Metcalfe & Ure (1995) and owing to the suitability of the zebra finch for a study on predation risk. As with most small bird species, wild zebra finches are taken by both avian and ground predators, mainly raptors, snakes and dingoes (*Canis familiaris dingo*) (Zann 1996), and although captive bred, no evidence of domestication is apparent in the zebra finches' behavioural responses to human contact. In attempting to compare alarmed and routine flights we were presented with the problem of gaining a large number of alarmed flights. To attain alarmed flights, Metcalfe & Ure (1995) presented birds with an artificial predatory stimulus when birds were on the ground. However this was carried out infrequently to prevent habituation, and as a result, insufficient data were collected for adequate analysis of individual flight performance. In order to get a larger sample size for comparison with the Metcalfe & Ure (1995) data set, we decided to capture birds, place them in a 10 cm high holding cup and alarm them upon release. Habituation was prevented by the continued negative reinforcement resulting from the bird's recapture.

Birds were housed in single sex pairs in cages (60 x 50 x 40 cm) and maintained on an artificial 14L:10D light regime, with surplus ad libitum mixed seed and water. We conducted flight trials in a separate vertical flight aviary (190 × 100 × 100 cm) where we released the birds from the floor, from where they flew vertically to a single perch mounted near the roof (see Fig. 1). To make the birds fly in as vertical a trajectory as possible, 40 cm high baffles were placed 20 cm apart either side of the release point at the base of the aviary (see Fig. 1). The baffle facing the observer was transparent, and the far baffle

was opaque and marked at a height of 40 cm from the ground so that the time taken to reach this point could be calculated. On each occasion that the birds were flown, they were first taken from their home cages, placed in a holding cup sufficiently wide (12 cm in diameter) to prevent undue restriction of the birds initial wing extension. The bird was then weighed whilst in the cup using a balance accurate to 0.1g (A&D Instruments EW 300G). The holding cup containing the bird was then placed in a sheath at the base of the flight aviary between the two baffles. The sliding lid of the cup was transparent, allowing the bird to observe the nature of the flight it was required to make prior to its release. The lid of the holding cup was then slid open, and simultaneously given a tap to scare the bird into flying out and up to the perch at the top of the aviary. The bird was then re-caught with a net, returned to the holding cup and allowed to recover for a minimum of 30 s. We familiarised individual birds with the flight aviary by flying them as they would be in the experiment until no further improvement in their flight speed was detected. This familiarisation period of approximately ten flights, (one flight every other day) was required to ensure birds were aware of the presence of the perch at the top of the cage to which they subsequently flew, and most importantly to ensure no improvement in the bird's physical condition occurred during the experiment which might affect flight velocity independently of body mass. An analysis of the first 10 training trials for all birds showed clearly that flight times did level off (linear regression: F_8=14.18, P=0.005, R^2=0.639). As the birds were housed in cages in which they could not perform extensive vertical flights, it was considered important to physically prime the birds prior to data collection. Subsequently no data for naive birds are included in any analysis, and thus the problems of habituation and the potential effect of improved physical condition were minimised.

We conducted trials over a period of two months, across a wide range of daylight hours from 0900 to 2000 in order to utilise the natural diurnal variation in body mass that is known to occur in zebra finches (Metcalfe & Ure 1995). Birds were flown on alternate days and a minimum of 15 flight trials (mean = 20.4) were recorded per bird. Each trial was composed of three

separate vertical flights. Thus regressions were based upon a minimum of 45 flights (mean = 61.2) for each of the 22 birds. While angle of take-off may also vary with mass (Witter *et al.* 1994; Kullberg *et al.* 1996), this parameter is not analysed here since it was not recorded by Metcalfe & Ure (1995), and so cannot be compared across flight categories.

As with Metcalfe & Ure (1995), we filmed flights using a camcorder (Panasonic NV MS 95B), and played the resulting video film through a digital video timer (For.A Video Timer VTG-33F) and re-recorded it on a VCR (Panasonic NV-FS90) so that digital time measures accurate to $1/100^{th}$ of a second were encoded on to each frame. This film was then played back on the VCR using a frame by frame facility to measure the time taken for each bird to fly vertically up to a line marked 40 cm from the ground. Times taken to complete the distance from the top of the holding cup, to the 40 cm mark were then converted into velocities and the mean velocity for each trial calculated from the three flights. The regression of this mean velocity against the corresponding body mass was then determined for each bird, as in Metcalfe & Ure (1995). We also tried quadratic regressions on the mass/velocity data, but this did not improve the relationships.

We then compared these linear regression equations with the original data used in Metcalfe & Ure (1995), calculated over the same distance from the ground, to establish the differences in the mass/velocity relationship between routine and alarmed flights. Metcalfe & Ure (1995) obtained data for both routine and alarmed flights. However, their sample size for the latter was relatively small for each individual bird and so most analyses in that paper were based on the routine flights. As the data in Metcalfe & Ure (1995) were collected using birds flying freely from the floor, certain corrections had to be made to enable direct comparisons with the present study where the birds were flown from a container. Flight times in the previous study were measured as the time from the moment when both legs and wings were fully stretched to the point when the bird's head had passed a mark 40 cm from the ground (Metcalfe & Ure 1995). During this time the bird would not have flown 40 cm, rather it would

have flown 40 cm minus the length of the bird stretched from toe to shoulder. To correct for this, five zebra finches were measured to give a mean stretched toe to shoulder length (X+SE =8.6 ±0.48cm, N=5). This was then subtracted from the 40 cm, and the flight velocities recalculated based upon this new distance (31.4 cm), the distance more realistically flown by the bird. We then re-calculated the individual regressions of velocity on mass based upon these revised distances. The Metcalfe & Ure (1995) data could then be compared with the data recorded in the current experiment, where velocity is recorded from the point when the head passes the edge of the 10 cm high cup (a height chosen to be equivalent to the stretched height of a zebra finch), to the point when the head passes a line 30 cm above that. Thus velocities for the two data sets were calculated over a comparable distance of 30 cm for the current experiment and 31.4 cm for the modified Metcalfe & Ure (1995) data set.

Having calculated the mass/velocity regressions for each bird, we then compared the slope component (b) of the equations for the different data sets using a one-way analysis of variance followed by a Tukey HSD multiple range test, or a T-test if there were only two groups. Comparisons of the probability or significance values (P), and the proportion of variation in velocity explained by mass (r^2), were made using the Kruskal-Wallis one-way analysis of variance test, followed by a multiple comparison test after Siegel & Castellan (1988), since these parameters were not normally distributed.

Results

To establish that the birds were not becoming habituated to handling, or improving during the flight trials, we regressed the mean flight time per trial for all birds flown over the first 15 trials (the lowest number of trials flown by any one bird) against trial number. No significant relationship was found (linear regression: $F_{1,3}$=0.517, P=0.487, R^2=-0.0357), indicating no evidence of habituation or improvement.

It is possible that alarmed birds might be more inconsistent in their individual flight velocities because of the onset of fatigue as the trial composed of three

flights progressed. In order to examine this, we considered the repeatability of the flight measures obtained. An analysis of variance for the calculation of repeatability (Lessells & Boag 1987), performed across the three flight measures recorded for all available trials showed the trials to be highly repeatable ($F_{2,2262}$=113.43, r=0.974), suggesting little inconsistency between the flights within a trial.

There were no significant differences in mean bird mass between the sexes or between the three data sets (Two way ANOVA: effect of experiment, $F_{2,32}$=2.121, P=0.117; effect of sex, $F_{1,32}$=3.179, P=0.055). In addition no significant differences were shown to exist between the sexes or between the three data sets in the variance of body mass over which the birds were tested (Two way ANOVA: effect of experiment, $F_{2,32}$=1.641, P=0.210; effect of sex, $F_{1,32}$=2.758, P=0.107). In the current experiment no significant differences were found in the relationship between mass and flight velocity of male (n=12) and female (n=10) zebra finches in terms of the regression slopes (t-test: t_{20}=0.17, P=0.87), their significance levels (Kruskal-Wallis one-way ANOVA: effect of sex, H_1=1.596, P=0.21), nor the extent to which variation in velocity was due to mass (r^2 values; Kruskal-Wallis one-way ANOVA: effect of sex, H_1=2.300, P=0.13). Therefore we felt justified in combining the data for the sexes in subsequent analyses of flight performance, so that they could then be compared with the combined Metcalfe & Ure data (1995).

Regression lines of flight velocity against body mass for each bird for the three sets of data (routine and alarmed from Metcalfe & Ure 1995, and alarmed from the present study) are shown in Fig. 2. A simple visual examination of the three graphs shows that the regression lines are more varied for alarmed flights in comparison to routine flights. Fig. 3 summarises a comparison between the three data sets. We aimed to compare not the exact positions of the different regression lines, but the strength of the mass/velocity relationships, as measured by the regression line slopes, their statistical significance and the proportion of variation in velocity explained by mass. We found differences in the values for the regression slopes across the three data sets (One-way

ANOVA, effect of treatment, $F_{2,36}$=7.74, P=0.0017). A Tukey HSD test located these differences as being between the slopes for the current data set and both data sets derived from Metcalfe & Ure (1995); (see Fig. 3a). The levels of significance (probability values) for the regressions also differed between the three data sets (Kruskal-Wallis one-way ANOVA: effect of treatment, H_2=19.22, P=0.0001). A multiple comparison test showed that differences existed between both alarmed data sets and the routine flights, with the regressions for alarmed flights having higher probability values (i.e. they tend to be non-significant) relative to those for routine flights (see Fig. 3b). In order to check that these differences in probability values of the regression lines were not simply due to differences in sample size, we examined the r^2 values of the regressions. Significant differences were found in the extent to which mass could explain the variance in velocity (r^2) between the three data sets (Kruskal-Wallis one-way ANOVA: effect of treatment, H_2=17.05, P=0.0002). A multiple comparison test showed that a significant difference existed between the alarmed data from the current experiment and the routine data from Metcalfe & Ure (1995). There was a clear trend for the r^2 values to be much lower in alarmed flights than in routine ones (see Fig. 3c), a result that is independent of sample size. Thus, it is evident that body mass was a poor (i.e. non-significant) predictor of flight velocity when birds were alarmed in both experiments, whereas it was a strong predictor when birds were making routine flights.

Discussion

The relationship between mass and alarmed flight velocity appears to be much weaker (if it can be said to exist at all) than the corresponding relationship for routine flights within the natural weight range of zebra finches. This may also be true in other birds. Witter *et al.* (1994), for example, found that adding weights of up to 8 g to starlings, equivalent to approximately 10% of body mass, did not significantly affect the alarmed flight velocity of the birds, although it did affect take-off angle. Kullberg *et al.* (1996) found that a 60% increase in fat load as a percentage of lean body mass in blackcaps affected angle of ascent but only resulted in a 17% decrease in the vertical alarmed

flight velocity. It should be noted that this regression line was based upon single flights for 28 birds of varying fat levels, rather than being calculated for individual birds. Data for alarmed flights appear to be in contrast to the routine flight data from Metcalfe & Ure (1995) where a mere 7% increase in mass was associated with a reduction of approximately 20% in routine vertical flight velocity in zebra finches. Metcalfe & Ure (1995) did not present data for individual birds for alarmed flights since the sample sizes for these were considered to be too small to give reliable results. Instead they presented a single regression of velocity on mass pooled across all birds. However, our much larger sample sizes (both of flights per bird, and number of birds tested) show that this pooled result is rather misleading since the relationship within individuals is weak, with mass having little or no effect upon velocity within the natural weight range of the bird.

It is possible that the restricted aperture of the holding cup could affect the flight performance of the birds by altering the air pressure distribution during the wing-beat at take-off. However, the diameter of the cup was chosen to be sufficiently wide to allow the bird to 'escape' without its wings hitting the edges of the cup, and the height of the cup was chosen so as to be close to the height of the bird at take-off. Subsequently, the down-flap of the wing-beat would occur sufficiently close to the aperture of the holding cup so that any restriction of airflow is likely to be minimal. Moreover, there is no obvious reason why such an alteration in airflow should be significantly different for the same bird at different masses. Thus this is unlikely to account for the lack of a significant relationship between mass and alarmed flight velocity.

One must then consider why birds that are capable of overcoming much of the apparently limiting constraints of mass upon flight performance when alarmed, do not do so when making routine flights, and why the relationship between mass and velocity is more variable when the birds are alarmed. There are numerous possible explanations, which are not necessarily mutually exclusive.

1. Alarmed flight is dependent upon the maximal power available to a bird, which is determined by the properties of the bird's muscles and skeleton, whereas routine flight is controlled by the maximum power continuously available, which is determined by the capacity of the animal to supply fuel and oxygen to the flight muscles (Pennycuick 1972). With such different determining factors for the different flight types, it is not surprising that different mass/velocity relationships are yielded.

2. For horizontal flight, the velocity which minimises energetic requirements is lower than the maximum velocity that the bird can achieve (Pennycuick 1989). This is because the power required to overcome parasitic drag increases with the cube of velocity. As parasitic drag caused by the air's resistance to the bird's body is likely to act in a similar way when a bird is flying vertically, we would expect the energy minimising speed for vertical flights also to be less than the maximum attainable speed. We suggest that birds performing routine flights will adjust their speed in order to save energy, but when alarmed will sacrifice energetic considerations for maximum speed. For example, in the corrected data derived from Metcalfe & Ure (1995) for which alarmed and routine data are available for each bird, we can see that on average each bird increased its velocity by 42% from the routine to alarmed condition (mean routine velocity = 1.77 m/s + SD 0.36, mean alarmed velocity = 2.51 m/s + SD 0.62). Given that the initial rate of climb is approximately constant from take-off over 40 cm (Metcalfe & Ure 1995), this would mean that the time taken to reach 40 cm from the ground would thus decrease from 0.23 s in the routine condition to 0.16 s for the alarmed condition. If birds making routine flights fly at a velocity which minimises their energetic costs, we would also expect them to fly slower as their mass increases (Witter & Cuthill 1993; Kullberg *et al.* 1996) as the metabolic requirements of flight are an accelerating function of body mass (Pennycuick 1972, Hedenström & Alerstam 1992). However, when under attack from a predator, birds should prioritise immediate survival above longer term energetic considerations, and so one might expect them to fly at the most effective (rather than the most efficient) velocity possible. The mass/velocity relationship would thus be flexible, and dependent upon the

prevailing conditions. Evidence supporting this comes from an analysis comparing alarmed flight velocities of each bird with the velocity predicted from a model that assumes the total power output (P_{max}) while flying was independent of its mass (see Pennycuick 1989, Hedenström & Alerstam 1992). The calculations were based on the procedure followed by Metcalfe & Ure (1995), and used their mean measure of zebra finch wingspan and Pennycuick's (1989) program 1A to calculate aerodynamic power. The actual alarmed velocities of the birds when at their maximum recorded mass were significantly greater (54.1% +13.5 SE) than those predicted if P_{max} had remained constant (Paired *t*-test, t_{21}=6.83, P<0.001), therefore when alarmed the birds did increase their total power output as mass increased.

3. Alarmed birds may be less consistent in their flights, possibly because of errors resulting from panic, or even deliberate evasive movements in flight trajectories made under the pressure of a potential predatory attack, thus lowering the r^2 values.

4. Birds performing routine flights may also reduce their velocity at higher individual masses to minimise the risk of injury from muscle strains as a result of flying faster or from the increased risk of collisions owing to their greater momentum, and consequently reduced manoeuvrability (Cuthill & Guilford 1990; Witter & Cuthill 1993). When escaping from predators, such considerations become relatively less important, and as such there is less of a reduction in velocity with increasing weight.

5. The mass/velocity relationship may be less marked in alarmed birds owing to variation in the perception of risk by the birds, which itself may be state dependent (Witter *et al.* 1994). For example when a heavier bird is alarmed, it may perceive its risk of capture to be higher, and may subsequently increase the effort in its escape flight, at a cost of, for instance, an increased risk of collision, lessening the apparent effect of mass. It is noticeable that the differences between alarmed and routine flights increased with increasing mass, owing to alarmed birds increasing their velocity relatively more when

heavy than when light (compare Figs 2a and 2c). This is supported by the fact that heavy alarmed birds were shown to expend on average 54.1% more energy than light alarmed birds. However, notwithstanding reasons 2 and 4, when under attack from a predator (apparent or otherwise), one might have expected all birds, not just heavier birds, to fly at a maximal velocity for their weight, and so the difference between light and heavy birds should be more substantial than it appears in Fig. 2c. It is possible, however, that there is a gradation in effort, where birds make progressively more strenuous attempts to fly fast as mass increases, which is supported by our results. Such a strategy of light birds flying at lower velocities than that of which they are capable even when under attack from predators, may evolve to ensure a more uniform take-off velocity within a flock. Such a strategy would benefit all members of the flock according to the selfish herd theory (Hamilton 1971) and by lowering the risk of collisions with other birds due to their more uniform velocity. By contrast, when performing routine flights, the bird's perception of risk will not be a factor, and subsequently the bird will not make an effort to overcome any potential limiting effects of mass. Although this experiment was carried out on individual zebra finches, zebra finches are a flocking species, so such a behavioural strategy may be innate, thus explaining its persistence when birds are isolated.

6. We found that mass had little if any effect upon alarmed flight velocity within the natural weight range of zebra finches (although 18 out of the 22 birds were faster when lightest than when heaviest as calculated from their respective regression equations). It is probable however, that above this natural weight range, any increase in mass would significantly diminish alarmed velocity. However, this natural weight range may have evolved as the range within which a bird can vary its weight without compromising its escape velocity, irrespective of the effect it has upon its routine flight velocity. In order to test this, one would have to increase the weight of birds and measure subsequent changes in velocity without the use artificial weights, which themselves may give rise to problems of increased parasitic drag, impaired wing movements, and an interference in the bird's centre of balance.

Considering these possibilities, it is perhaps not so surprising that the relationship between mass and velocity for alarmed flights is much weaker than those for routine flights within the bird's natural weight range. This is consistent with the view of Witter & Cuthill (1993) and of Kullberg *et al.* (1996), that birds are not flying at their maximum velocity for a given weight when unalarmed. We suggest that the extent to which mass affects alarmed take-off performance within a bird's normal weight range may be marginal in terms of velocity, if not for other costs such as energy expenditure, collision risk or other parameters such as take-off angle (Witter *et al.* 1994) and manoeuvrability (Metcalfe & Ure 1995). Consequently the use of routine flights to investigate the effects of mass upon predation risk may tend to over-estimate the potential predation risk of elevated body mass. Previous studies on the effects of mass upon routine flight velocity may have highlighted behavioural/physiological trade-offs rather than absolute physical constraints upon flight performance. In order to translate the more marginal effects of mass upon alarmed flight velocity into potential fitness costs, research should focus upon calculating the extent to which mass affects alarmed flight velocity, take-off angle and manoeuvrability, and just how much these changes affect predation risk, and other costs of flight.

Fig. 1.

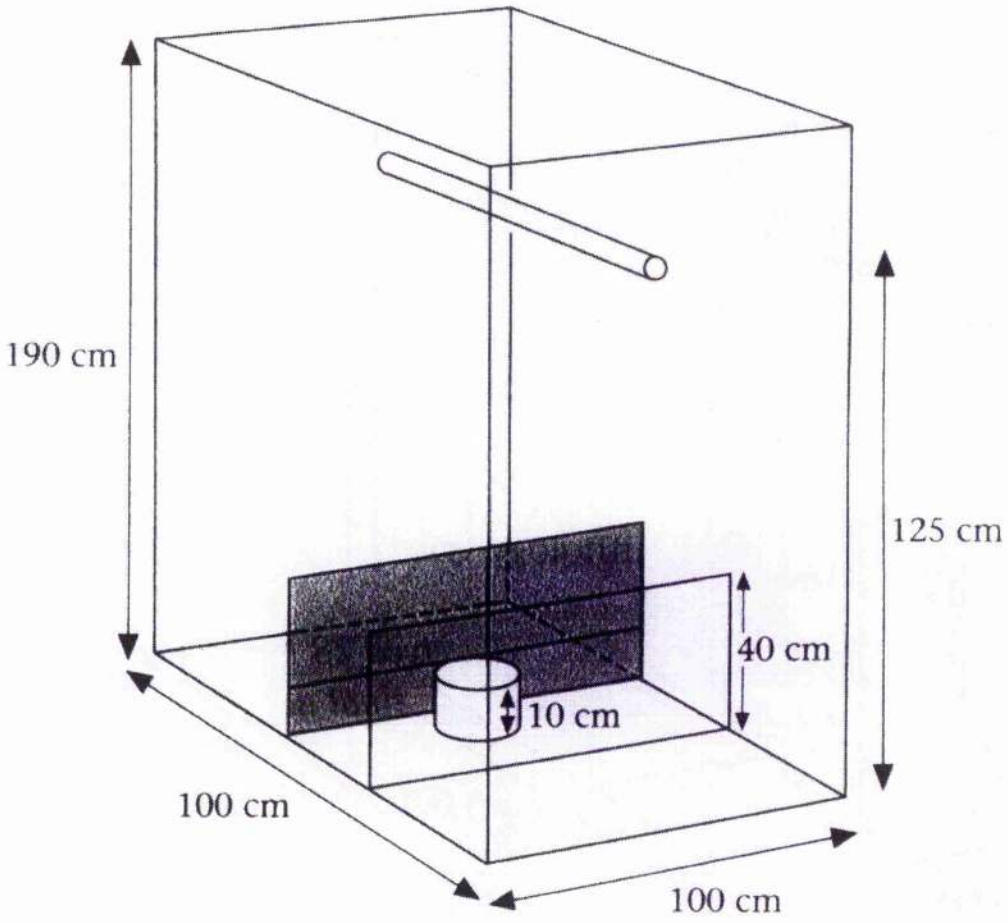


Fig. 1. Vertical flight aviary showing the 12 cm wide and 10 cm high holding cup in the centre from where the birds were released, flanked by an opaque and transparent baffle. Birds flew vertically upwards from the holding cup to the perch positioned 125 cm from the base of the aviary.

Fig. 2.

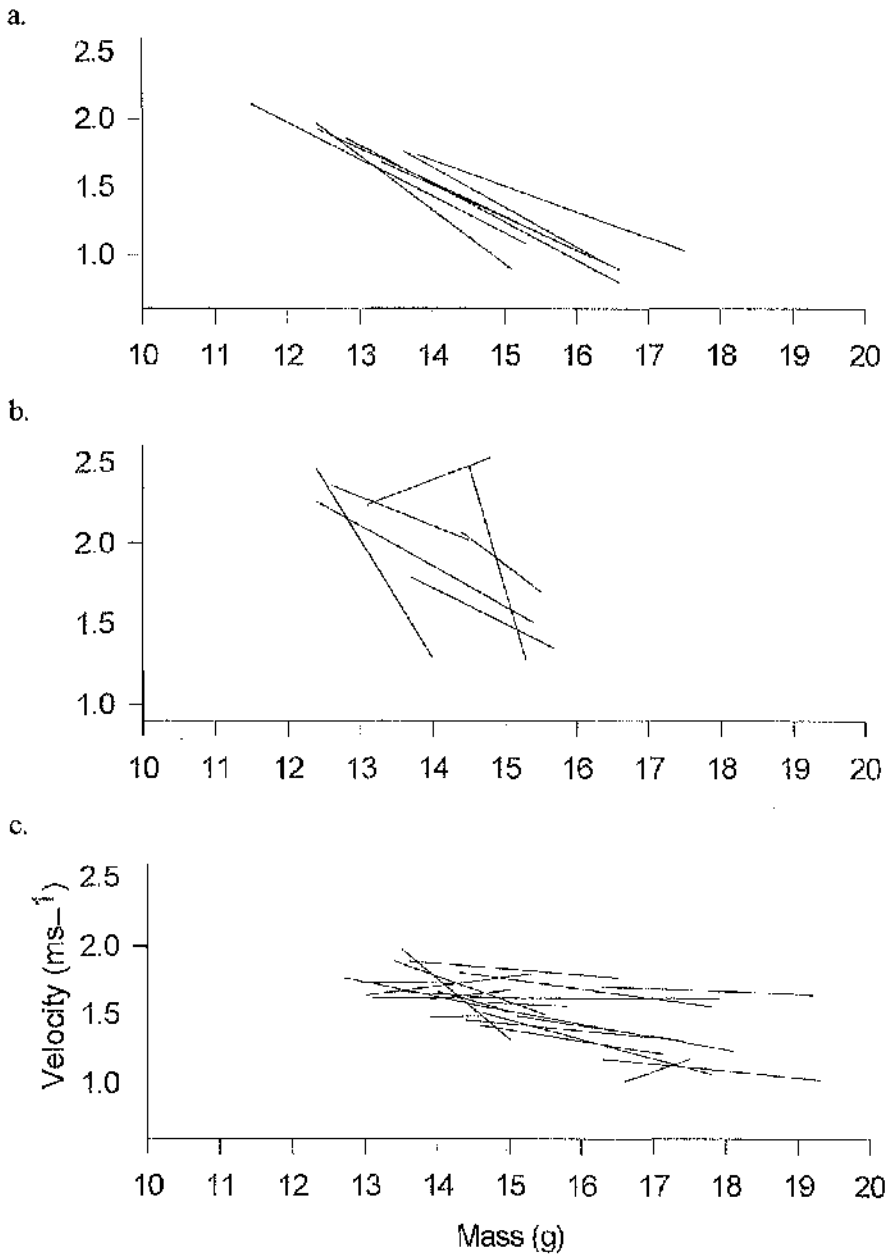


Fig. 2. Relationship between body mass and flight velocity over a vertical height of 40 cm from the ground in individual zebra finches. (a) Routine flights derived from Metcalfe & Ure (1995); n=8 birds; (b) alarmed flights derived from Metcalfe & Ure (1995; n=7), (c) alarmed flight from current experiment (n=22).

Fig. 3.

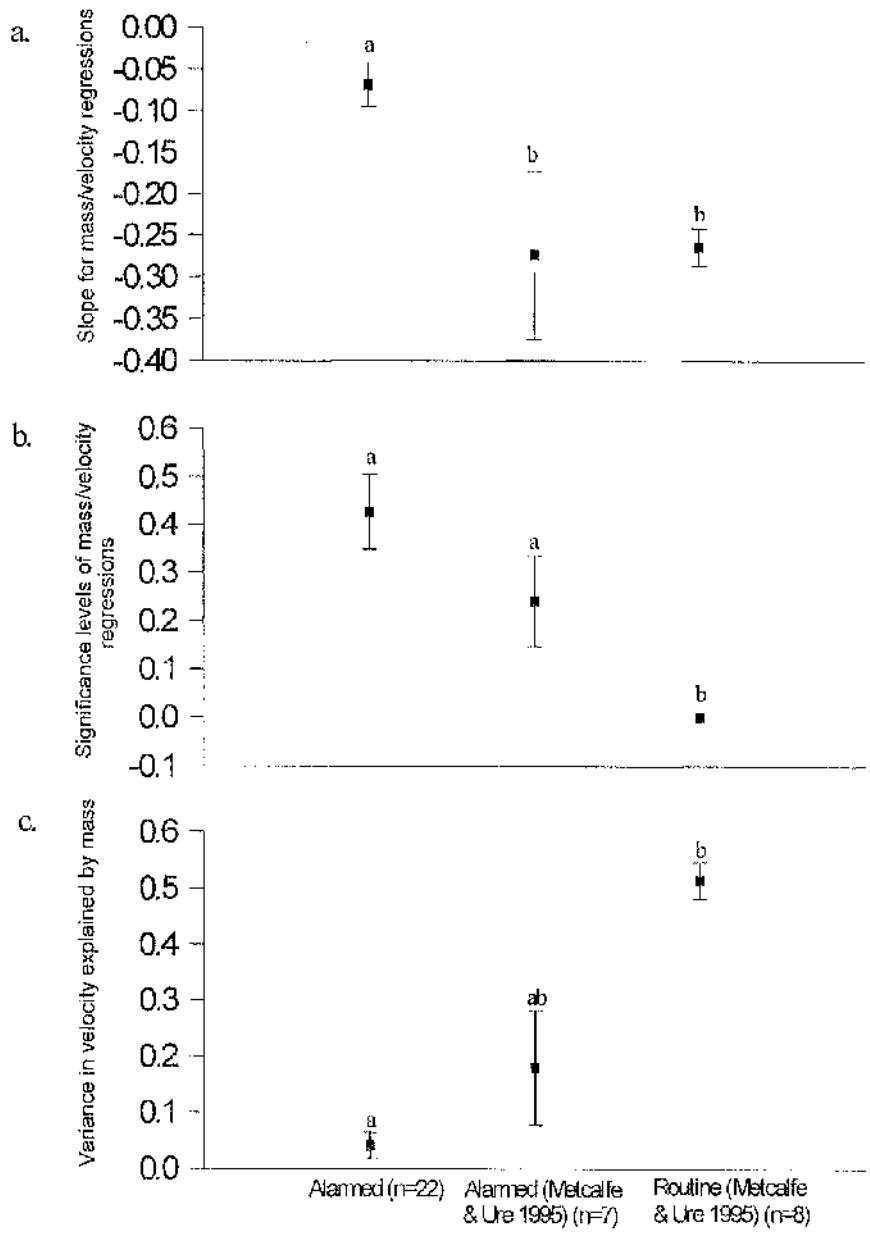


Fig. 3. Summary of mass/flight velocity regression equation components, (a) regression slope values, (b) Significance levels, (c) variance in velocity explained by mass (r_2). Bars with different letters indicate those that were significantly different (see text for statistical analyses).

Chapter 3

Flight muscle atrophy and predation risk in breeding birds

Abstract

Take-off velocity is critical to the escape response of most small birds when attacked by a predator. However, take-off ability is likely to be effected by physiological changes occurring as a result of egg formation. To investigate whether reduced take-off velocity is a cost of reproduction, we experimentally manipulated the physiological costs of egg production in the zebra finch (*Taeniopygia guttata*) by varying the number of eggs a female laid and the quality of her pre-laying diet. We subsequently measured the effect of changes in post-laying flight muscle condition and body mass upon alarmed flight performance; a measure of predation risk in birds. Changes in muscle condition were found to correlate with changes in various measures of flight performance: Treatments which caused the greatest declines in muscle condition during laying were associated with the greatest declines in flight performance over this period. In contrast, those laying attempts which caused the least declines in muscle condition were associated with improvements in flight performance (i.e. birds flew faster at the end of laying than at the start). These effects were independent of changes in body mass, and occurred post-laying, suggesting that egg production itself is costly rather than carrying eggs as other studies had suggested. The observed negative trade-off between muscle loss resulting from egg production and escape ability could have important implications on the evolution of optimal clutch size in birds.

Introduction

One of the main assertions of life history theory is that reproduction is costly, and current reproductive effort can diminish the chances or amount of future reproduction (Stearns 1992). However, the underlying mechanisms of how reproduction affects survival are poorly understood (Tatar & Carey 1995). The majority of studies (96.9%) investigating the costs of reproduction in birds have in the past emphasised the post-laying phase and so may have missed

costs occurring earlier in the breeding cycle (Monaghan & Nager 1997). This emphasis on post-laying costs may have come about for two reasons. Firstly it is easier to manipulate the number of chicks reared than it is to manipulate the number of eggs laid. Secondly, in the past, egg production has been considered as inexpensive due to the apparent ease with which birds replace lost eggs or even entire clutches (Monaghan & Nager 1997). However, more recently, evidence is accumulating that egg production is costly in terms of energetic expenditure (Ward 1996), female survival (McCleery *et al.* 1996), subsequent breeding success (Heaney & Monaghan 1995, Monaghan *et al.* 1998) and muscle condition (Kendall *et al.* 1973, Jones 1991, Houston *et al.* 1995abc Monaghan *et al.* 1998).

Recent work has shown that a wide range of bird species (21 out of the 29 for which muscle volume changes have been studied) exhibit declines in muscle condition during the laying period (Houston *et al.* 1995a). It has been suggested that these declines represent a direct contribution of muscle proteins to egg production (Kendall *et al.* 1973, Jones & Ward 1976, Houston *et al.* 1995bc). Birds can be classified as capital breeders relying upon stored nutrients, such as those which might exist within the flight musculature, or income breeders relying upon daily dietary intake for egg formation (Drent & Daan 1980). It is typically assumed that small passerines such as the zebra finch must be income breeders because clutch masses often represent a high proportion of the female's body mass. However, it has been shown that in the female zebra finch, muscle proteins are transferred directly to eggs during their formation (Houston *et al.* 1995bc), so that females may subsequently undergo an average reduction of 14% in protein form the pectoral muscles (Houston *et al.* 1995a) despite the availability of ad libitum food during the laying period.

Similar results have been reported for the red-billed quelea (*Quelea quelea*), which have been shown to lose 16 % of their muscle mass during breeding despite an abundance of food. This loss of muscle condition is roughly equivalent to being half way between the peak condition ever recorded for this species, and the condition of birds which have died of starvation (Kendall *et al.*

1973, Jones & Ward 1976). Costs associated with such a substantial depletion of protein reserves are likely to be particularly high if the proteins are derived from contractile muscle fibres involved in flight. It has already been shown that the flight muscle ratio (flight muscle mass/body mass) positively correlates with residual lift capacity (Marden 1987), and so one might expect a reduction in foraging efficiency (see Heaney & Monaghan 1995) or alarmed flight velocities to occur as a result of reproduction in capital breeders. Since getting off the ground rapidly and staying above pursuing predators is critical in surviving attacks from mammalian and avian predators (Rudebeck 1950, Howland 1974, Page & Whitacre 1975, Cresswell 1993, Bednekoff 1996), predation risk in birds can be assumed to increase if the animal's alarmed flight speed has been reduced. Changes in flight muscle condition might therefore be expected to profoundly affect maternal survival probabilities, particularly in relation to predation risk.

Studies attempting to demonstrate a link between reproductive output and subsequent maternal predation risk have typically focused upon the increased likelihood of a predatory attack rather than the increased risk once an attack has been initiated (Magnhagen 1991, Székely *et al.* 1994) or have highlighted the decline or cessation in reproductive activity of animals under increased risk from predation (Korpimaki *et al.* 1994, Magnhagen 1991). The importance of predation on parents as a cost of reproduction may have been unappreciated in the past, since most detailed studies have taken place in the northern temperate zones where the effect of predators upon adult birds has been greatly reduced by the activities of man in removing predators from ecosystems (Lima 1987a). It should also be noted that clutch size manipulations themselves tend to decrease predation risk due to the continued presence of humans and the use of predator-proof nest boxes (Stearns 1992). However, since predators have begun to return to near historical levels, the importance of predation upon adult survival, and subsequently evolution, has begun to be appreciated more (see Gosler *et al.* 1995, Slagsvold & Dale 1996).

A number of studies have looked at the effects of reproduction upon predator vulnerability once an attack has been launched, but these have typically focused upon increases in mass associated with reproduction in flying animals (Jones 1987, Hughes & Rayner 1993). However, recent work has shown that within the natural weight range of a bird, mass may not be significant in effecting alarmed flight velocity over short distances during take-off (Witter *et al.* 1994, Lee *et al.* 1996, Kullberg *et al.* 1998, Kullberg 1998, Veasey *et al.* 1998, Lind *et al.* in press). Although a number of studies investigated the effect of egg carrying upon locomotion (Berglund & Rosenqvist 1986, Schwazkopf & Shine 1992, Lee *et al.* 1996), I am not aware of any demonstration that the consequences of egg production (rather than of carrying eggs) may increase predation risk in iteroparous animals. In an attempt to redress the balance of previous studies, this study will focus upon the costs of egg production rather than chick rearing, and follow on from the work of Houston *et al.* (1995abc) which found that flight muscle condition declines during laying in zebra finches. The aim of this investigation therefore, is to discover the extent to which the maximal flying ability of females, and hence their ability to escape from predators, is affected by losses in muscle condition resulting from laying. To do this trials were run in which the same group of birds was allowed to lay a succession of clutches. The extent of the change in muscle condition during each breeding attempt was varied by manipulating the number of eggs laid and the quality of the bird's pre-breeding diet. The relationship between the changes in post-laying flight performance and flight muscle condition was then examined.

Methods:

Treatments

Previously established zebra finch pairs were housed in separate cages (60 x 50 x 40 cm) and maintained on an artificial 14L:10D light regime throughout the experiment. Four treatments were utilised in order to manipulate changes in female flight muscle condition resulting from egg laying. These treatments consisted of manipulations of pre-breeding diets and clutch sizes. The two possible pre-laying diets were a low protein diet of ad libitum mixed seed, and

a high protein diet of ad libitum mixed seed with a high quality protein supplement containing soya protein and homogenised boiled hen's eggs (Selman & Houston 1996a). This high quality protein diet is known to reduce the extent to which female zebra finches draw upon their pectoral muscles when producing a clutch (Selman & Houston 1996a). The two clutch manipulation treatments encouraged the laying of relatively large or small clutches: removal of the first four eggs on the day each was laid induced the laying of a larger clutch, while adding one false egg daily to the nest box for four days, starting four days after pairing, induced birds to lay smaller clutches (see Haywood 1993ab). These two manipulations were combined to make four treatments which manipulated both the clutch size produced and the nutritional cost of producing a given clutch (see Table 1). In all trials birds were given free access to water, grit and cuttlebone. The sexes were kept apart until the breeding portion of each trial was started, at which point pairs were reunited, cages provided with nest building material and nest boxes, and all diets reverted to the low protein type for the duration of the laying attempt. All eggs were removed from the nest box on the day of laying, weighed and replaced with an artificial egg where appropriate.

Once all the birds had completed their clutches (when two days had passed without an egg being laid), the nest boxes were closed and the sexes separated again. A period of four to six weeks was allowed for recovery before the onset of the next breeding attempt, during which time the birds were maintained on the appropriate pre-breeding diet for the following trial.

Flight performance

Flight performance was assessed by recording the time taken to complete vertical flights when alarmed, since such escape flights are a better indicator of changes in predation risk than unalarmed flights (Veasey *et al.* 1998). Females were tested for flight performance prior to re-uniting the pairs, so as to obtain a measurement of their pre-laying flight performance (see Veasey *et al.* 1998 for details). Once pairs had been re-established, females were flown only on the days on which they laid an egg. Flights were conducted in a separate vertical

flight aviary (190 × 100 × 100 cm), with 40 cm high vertical baffles positioned 20 cm apart either side of a holding chamber at the base of the aviary, in order to make the birds fly in as vertical a trajectory as possible. Although body mass has been shown to have only a minor effect if at all on alarmed flight velocity in the zebra finch (Veasey *et al.* 1998), females were flown at a time when their body mass was known to be most stable, that is to say between three to five hours after dawn (Dall & Witter 1998), in order to minimise any confounding effects of the natural diurnal variation in body mass that is known to occur in the zebra finch (Metcalfe & Ure 1995, Dall & Witter 1998).

On each occasion that a bird was flown, it was first taken from its home cage, placed in a holding chamber and weighed to the nearest 0.1g. The holding chamber containing the bird was then placed in a sheath at the base of the flight aviary from where it was then startled into making a vertical escape flight toward a perch at the top of the aviary. The bird was then re-caught, returned to the holding chamber and allowed to recover for a minimum of 30 s. before the procedure was repeated. Each test on a given day consisted of recording three vertical flights for each bird. The measures obtained from these replicate flights have been shown to be highly repeatable (Veasey *et al.* 1998). In order to minimise the stress subjected to the birds, the flight trials were made predictable by the accompaniment of an audible stimulus, in the form of the playing of a radio. Thus birds were only ever handled when the radio was playing, and although this increased the levels of agitation amongst all birds for the short period when the radio was playing (approximately two hours in every 24 hour period), the birds were visibly more relaxed whenever the radio was not playing.

Flights were filmed using a camcorder and played back on a VCR using a frame by frame facility to measure the time taken for each bird to fly a vertical distance of 15, 30 and 115 cm from the top of the holding cup. Mean values from the three flights were then calculated for each bird, for each of the defined heights on each day on which the bird was flown.

Muscle condition

Since the changes in body mass during the course of the laying period will reflect changes in the reproductive tissues within the bird, as well as the presence or absence of eggs, 'body mass' will not give a satisfactory measure of female condition. However, mass changes are still recorded since there is a large body of evidence which suggests that mass will affect flight performance (for examples see Hartman 1965, Pennycuick 1969, Metcalfe & Ure 1995). As an alternative measure of condition, female flight muscle condition is considered. Although leg muscle condition has been shown to decline in laying female zebra finches (Houston *et al.* 1995c), and leg musculature will be important in take-off performance (Marden 1987), this is not considered here for two reasons. Firstly, it would not be possible to adequately monitor changes in the leg musculature of laying female zebra finches, and secondly, an analysis of the data from Houston *et al.* (1995) on changes in flight and leg muscle mass during laying, showed there to be a strong correlation between the two (Pearson's correlation: $r=0.97$, $n=7$, $P=<0.001$).

To investigate changes in female flight muscle 'condition' associated with reproduction, the size of the pectoral muscles of females were measured using a technique modified from Selman & Houston (1996b), females were measured both immediately prior to being reunited with their mate (to give a pre-laying measurement), and on the completion of the clutch (two days after the last egg was laid). Individual birds were placed breast downwards into a 2 cm deep tray of dental alginate (Cavex CA37 Superior Pink), which gives a faithful body mould without adhering to feathers (Selman & Houston 1996b). Two people were required for this procedure, one to hold the head, tail and the legs clear from the gel, while the other kept the wings from flapping. The alginate sets approximately one minute after mixing with warm water. Birds were placed in the gel approximately 45 seconds after mixing, so that they were held in position for as short a time as possible. The birds were then eased from the mould which was subsequently cut with a blade dorso-ventrally at the mid-point between the fulcra and the posterior portion of the sternum (figure 1a).

The cut surfaces of both halves of the mould were then placed in a plastic tray to maintain their rigidity, and pushed downwards onto an ink pad. Ten prints (five from each half of the mould) were then taken of the cross sectional profile of the pectoral region (figure 1b). This was carried out immediately upon the setting of the alginate to avoid errors resulting from shrinkage of the moulds. A horizontal line was then drawn 5 mm perpendicular from the base of the print (approximately the average keel depth size in zebra finches) corresponding to where the keel would have been at its deepest (figure 1b). The enclosed area was then measured using a computer plotter (BBC Master computer with cherry digitiser and puck), and a mean of these muscle cross-sectional areas taken of measures from five separate prints, at least two being from each half of the mould. An analysis of variance for the calculation of repeatability (Lessells & Boag 1987) performed across the five measurements confirmed that the repeatability was high ($F_{19,99}$=17.825, r = 0.771). The mean cross sectional pectoral muscle area is hereafter referred to as the muscle condition index.

All treatments outlined in Table 1 were repeated twice (with the exception of treatment 4, i.e. the high protein pre-breeding diet with the addition of eggs, which was only run once), in a sequence alternating from low to high protein pre-laying diets to avoid any longer term effects of continued high protein supplementation (see Williams 1996). The percentage change in muscle condition index, from pre to post-breeding was calculated for each breeding female, along with the corresponding mean percentage change in flight times (for each of the flight parameters) between the day of the first egg and that of the last egg. All statistical analyses are based on using one mean value for all females per trial (n=7 trials) to avoid problems of pseudoreplication.

Results

For each of the three flight parameters (flights to 15, 30 and 115 cm), linear regression analysis was used to examine whether the changes in muscle condition index during breeding influenced changes in flight times between the first and last egg across the seven breeding trials. Changes in muscle condition

index produced significant changes in flight times to 15 cm ($F_{1,5}$=12.614, adjusted R^2=0.659, P=0.016, B=-1.450±0.408, [figure 2a]), to 30 cm ($F_{1,5}$=25.383, adjusted R^2=0.803, P=0.004, B=-0.962±0.191, [figure 2b]), and to 115 cm ($F_{1,5}$=7.520, adjusted R^2=0.521, P=0.041, B=-1.198±0.437, [figure 2c]).

If breeding caused only a small change in pectoral muscle condition then birds tended to fly faster at the end than at the beginning of egg laying. However if the experimental treatment caused the birds to lose a larger amount of muscle condition, then their flight performance deteriorated during the laying period (figures 2a, 2b, 2c). The incorporation of changes in body mass into these analyses did not improve the models for the shorter two flight distances of the three, but did for the 115 cm flight times (multiple linear regression: $F_{2,4}$=16.822, adjusted R^2=0.841, P=0.011, B=-0.970±0.261), though changes in body mass alone were not significant over this height (linear regression: $F_{1,5}$=5.570, adjusted R^2=0.432, P=0.065). In order to correct for the fact that the three flight measurements were not independent, Bonferroni correction can be used (where P=<0.05/3). Results for 15 and 30 cm are still significant, as are the 115 cm results if changes in mass are incorporated into the model. The results demonstrate that the mean change in muscle condition index during breeding tended to be the most significant factor affecting the change in flight times during the course of a breeding attempt, particularly for the immediate take-off measures, while changes in body mass were generally unimportant.

Mass tended to have an opposing effect to the changes in muscle condition index over 115 cm (compare figures 2c with 3a). Therefore when the mean change in flight performance over 115 cm is corrected for mass using the mean mass/flight time slope derived from a substantial pre-breeding data set previously collected (see Veasey *et al.* 1998), the effect of changes in muscle index becomes even stronger (linear regression: $F_{1,5}$=15.517, R^2=0.756, P=0.011, B=-0.977±0.248 see figure 3b).

Discussion

It has previously been stated that 'in a class of vertebrates in which the young depend on healthy parents for food and protection, protein loss from muscles can not normally be so great as to impair locomotion' (Jones 1991). In this series of experiments, it has successfully been demonstrated that this is not the case, with muscle wastage resulting from egg production being sufficient to decrease alarmed flight speeds to the extent that predation risk may be increased. In all three measures, the difference between the best and the worst of the changes in muscle condition resulted in a variation of approximately 20% in the time taken to fly the defined trial distances. A number of studies investigating the effects of reproduction upon locomotion in birds (Lee *et al.* 1996), reptiles (Schwarzkopf & Shine 1992) and crustacea (Berglund & Rosenqvist 1986) have primarily considered the costs of carrying eggs. Lee *et al.* (1996) claimed they could not determine the relative importance of the production of eggs and the carrying of them in affecting flight performance. That the changes in flight times here appear to be independent of changes in body-mass, particularly at the take-off stage, and are seen to persist into the post-laying phase, indicates that the real cost may lie not in the carrying of developing eggs, but rather in the production of eggs and the muscle wastage that this incurs. These results demonstrate not only the existence of a cost of egg production, but also the underlying mechanism of that cost. The implications of this are wide reaching, and suggest that post-laying maternal predation risk may form part of a physiological trade-off between reproduction and survival, where investment in developing eggs competes directly for resources vital to the maintenance of flight muscle tissue. These findings have implications not only upon maternal predation risk, but also maternal foraging efficiency. The widespread nature of courtship feeding may be an attempt to minimise the exposure of the female to predation at a vulnerable time, or may be an attempt by the male to compensate for the reduction in female foraging efficiency (see Lee *et al.* 1996). Krebs (1970) for example, found a reduction in foraging efficiency of blue tits (*Parus caeruleus*) prior to laying, potentially resulting from the mobilisation of muscle tissue, and the hypertrophy of reproductive organs. Such costs of reproduction may be sufficient to depress

clutch sizes below the maximum which parents are capable of rearing, especially since it has been shown that changes in muscle condition are proportional to the number of eggs laid (Monaghan *et al.* 1998, see Chapter 5). In an environment where predators are present, females must balance gains in fecundity with losses in muscle condition and the subsequent increase in predation risk that this may entail. In Chapter 5, it is demonstrated that an increase in clutch size of just over one egg resulted in an increase in flight times of 7% at take-off, which is sufficient to allow a pursuing raptor to travel between 70-120 cm toward the escaping bird. Such a reduction in velocity would greatly reduce the chances of survival once attacked (Rudebeck 1950, Page & Whitacre 1975, Cresswell 1993, Bednekoff 1996).

Lack (1947) was the first to propose that clutch size may have evolved to an optimal level, whereby birds will lay a clutch which gives rise to the most young fledged. He hypothesised that as brood sizes increased, each chick would receive less resources and subsequently survive less well (Lack 1947), and so clutch size was for a long time considered to have been determined by the trade-off between the number and fitness of offspring rather than by any costs to the parent. This was apparently corroborated by the work of Pettifor *et al.* (1988), who found the addition or subtraction of hatchlings to a nest reduced recruitment in great tits (*Parus major*), but did not affect parental survival, thus leading to the assumption that reproduction itself was not costly. However, the existence of parental costs are suggested by the fact that the mean clutch size in many birds appears to be consistently lower than the most productive clutch size (Stearns 1992). The trade-off between reproduction and predation risk is such that a marginal increase in fitness such as laying an extra egg, or laying a clutch when in a poorer nutritional state, may jeopardise a female's entire residual reproductive value (Clark 1994). Based upon life expectancy in the wild (51 days at hatching), and the number of breeding attempts made per season (1.7 ± 0.9) (Zann 1996), it is apparent that future reproduction may be a relatively small proportion of the life time reproductive success in the zebra finch compared to other bird species. Consequently, zebra finches may be more tolerant to reductions in flight performance (and hence to

increases in predation risk) when compared to longer lived species, or species that make more breeding attempts in their life whilst at a similar risk of predation. We would therefore expect longer lived species to be more conservative when deciding whether or not to breed under the risk of predation.

It is likely that females would attempt to minimise any increases in predation risk by behavioural adjustments at the time of laying. It has been shown for example that activity in female birds declines at the time of laying (Houston *et al.* 1995c). This phenomenon has previously been interpreted as attempts to protect developing eggs or to minimise energetic expenditure (Fogden & Fogden 1979, Houston *et al.* 1995c). However, Schifferli (1976) found that developing house sparrow (*Passer domesticus*) eggs were not vulnerable to mechanical damage during normal activity, and in this series of experiments in which 37 females were flown on 2,505 occasions, not a single individual showed symptoms of having damaged an egg. It seems unlikely that the observed reduction in female activity at this time is a result of an attempt to minimise energetic costs during laying as it can be argued that one might expect an elevation in activity to allow for the increased intake of resources needed for the reproductive attempt. I suggest that the decline in activity is more likely to be an adaptive response to increased predation risk resulting from muscle wastage, whereby females deliberately reduce their exposure to predators at the time of laying. Thus I would expect females to adjust those behaviours which influence their predation risk most (see Chapter 7).

Table 1.

	Low protein pre-breeding diet	**High protein pre-breeding diet**
Four eggs removed from clutch	**Treatment 1:** Female induced to lay large clutch on poor quality diet.	**Treatment 2:** Female induced to lay large clutch on high quality diet.
Four eggs added to clutch	**Treatment 3:** Female induced to lay small clutch on poor quality diet.	**Treatment 4:** Female induced to lay small clutch on high quality diet.

Table 1. This table demonstrates how the manipulation of two factors, i.e. clutch size and diet quality are combined to give rise to four treatments.

Fig. 1a.

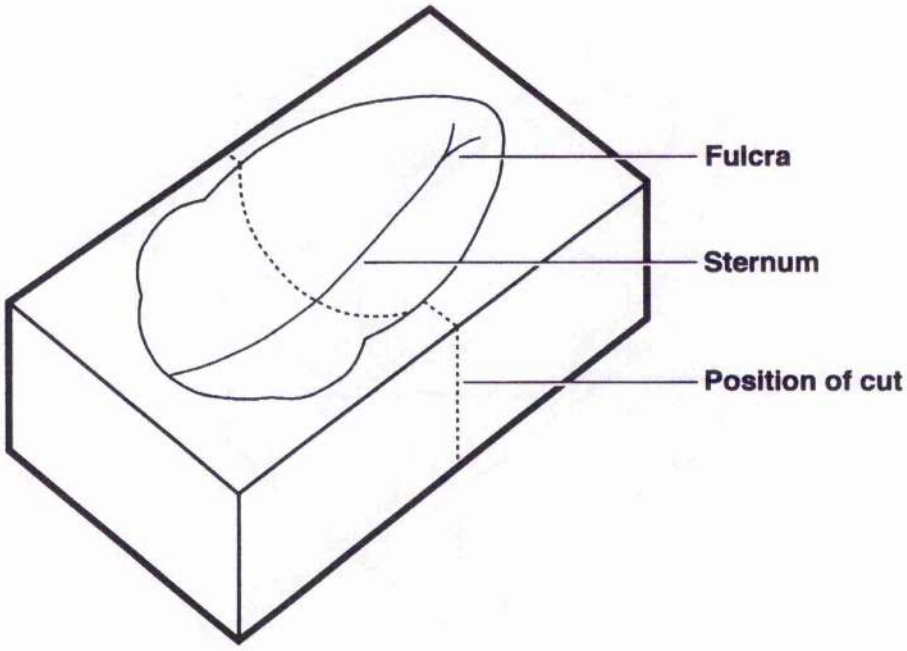


Fig. 1a. Schematic representation of an alginate mould taken from the pectoral muscle area of a zebra finch showing the position where the mould is cut, and prints consequently taken.

Fig. 1b.

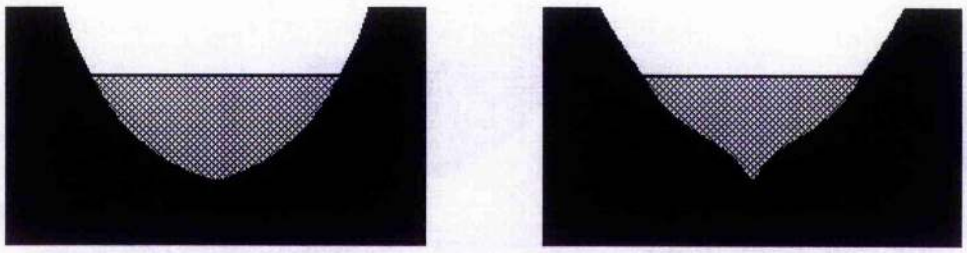


Fig. 1b. Schematic representation of prints taken from alginate moulds with the area measured; the muscle condition index, shown shaded. The right hand print is typical of a post-breeding female showing the pronounced keel, whereas the left hand print is typical of a pre-breeding female.

Fig. 2.

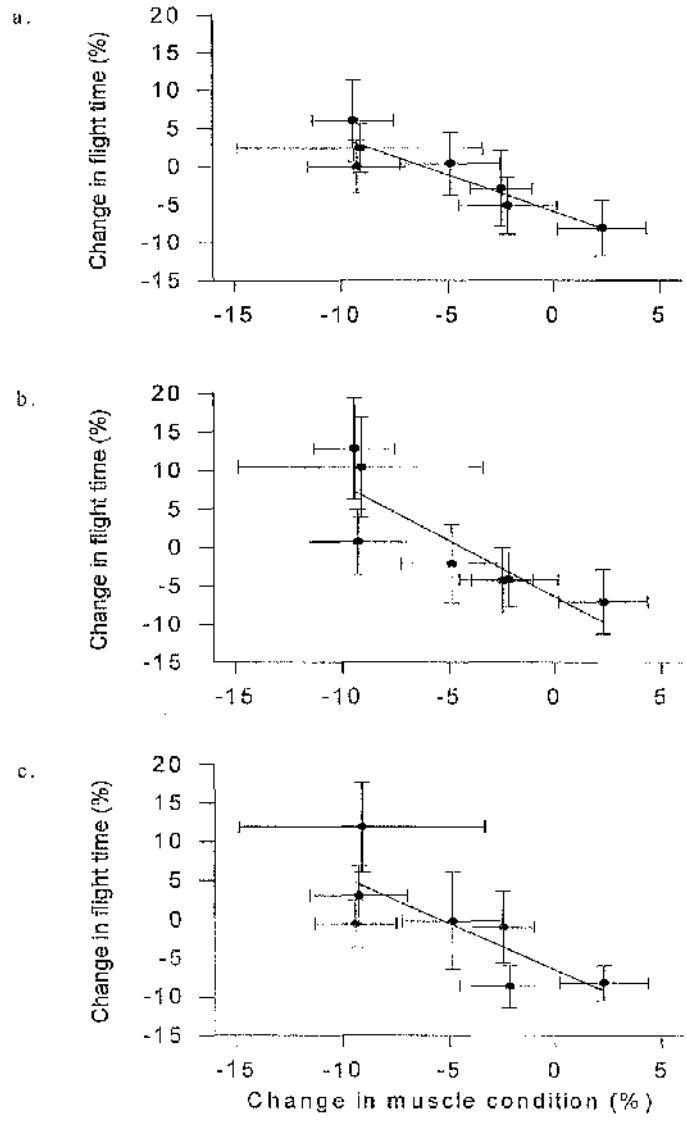
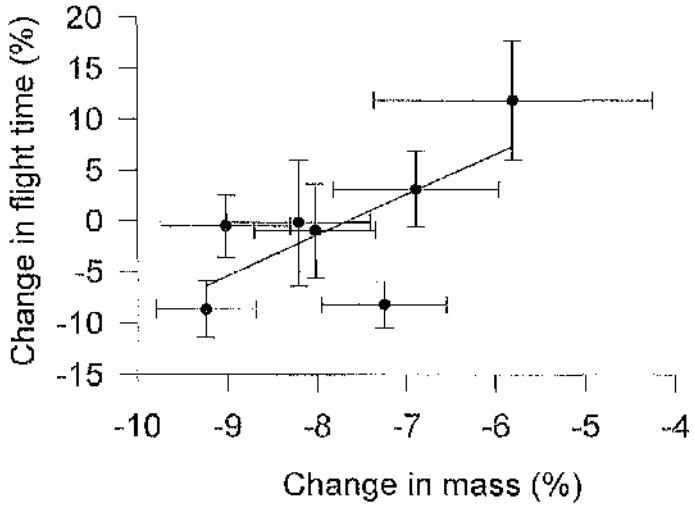


Fig. 2a. The relationship between mean changes (±SE) in female muscle condition index during the course of laying a clutch with the mean changes (±SE) in flight time over a height of 15 cm between first and last eggs. Fig. 2b. The relationship between mean changes (±SE) in female muscle condition index during the course of laying a clutch with the mean changes (±SE) in flight time over a height of 30 cm between first and last eggs. Fig. 2c. The relationship between mean changes (±SE) in female muscle condition index during the course of laying a clutch with the mean changes (±SE) in flight time over a height of 115 cm between first and last eggs.

Fig. 3.

a.



b.

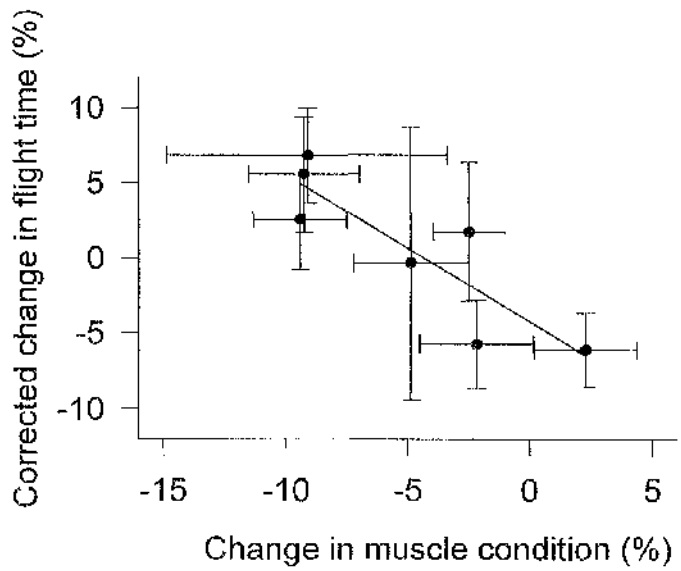


Fig. 3a. The relationship between mean changes (±SE) in female body mass during the course of laying a clutch with the mean changes (±SE) in flight time over a height of 115 cm between first and last eggs. Fig. 3b. The relationship between mean changes (±SE) in female muscle condition index during the course of laying a clutch with the mean changes (±SE) in flight time over a height of 115 cm between first and last eggs corrected for changes in body mass.

Chapter 4

A cost of protein storage in the flight muscles of zebra finches

Abstract

Reproduction is known to be costly in all groups of animals. Costs associated with reproduction can occur at all stages of the reproductive cycle. In the course of this experiment evidence is provided that reproduction in the zebra finch (*Taeniopygia guttata*) may be costly to females, even before the onset of laying. It is shown that the relative size of flight muscle tissue is larger in female zebra finches than it is in males, supporting the hypothesis that females may store proteins which are used in the production of eggs. Moreover, it is demonstrated that such protein storage may be costly since females with larger flight muscle indices tend to fly slower than those with smaller flight muscles, independently of weight. The costs associated with protein storage, must thus be weighed against the potential benefits associated with protein storage such as enlarged clutch size and quality and the maintenance of flight performance at the end of laying.

Introduction

Egg production represents a nutritionally and energetically demanding time for female birds. Robbins (1981), for example, estimated that the daily protein requirements for egg formation varies between 86-230% of normal daily requirements depending upon species and egg size, and Walsberg (1983) estimated the peak daily energetic costs during egg production to vary between 37-230% of basal metabolism depending upon species. To satisfy these requirements, some bird species appear to have evolved a strategy reliant at least in part, upon the utilisation of stored nutrients for the formation of eggs (Houston *et al.* 1995a, Jones 1991, Kendall *et al.* 1973), while others obtain nutrients needed for egg formation from their diet (Drent & Daan 1980). Jones & Ward (1976) proposed that some birds may use the level of protein reserves in their muscles as a threshold to initiate breeding and showed that in red-billed quelea (*Quelea quelea*) there was a substantial loss of muscle mass during the

days in which eggs were formed. Since this study, 21 species of bird out of 29 where changes in muscle condition have been studied, have demonstrated significant declines in muscle mass (Houston *et al.* 1995a). In zebra finches, females lose approximately 14% of their muscle mass during the course of egg formation (Houston *et al.* 1995c), and the use of labelled amino acids has shown that muscle proteins are used to contribute directly to egg production (Houston *et al.* 1995b). It has since been shown that these changes in muscle mass have implications for the flight performance of the female after laying (Chapter 3). In this chapter, one further aspect is considered in detail, namely the storage of proteins by birds, and the potential costs of such storage.

Kendall *et al.* (1973) proposed that bird muscle may contain a labile component which serves as a protein reserve which could be lost without impairing the contractile performance of the muscle. Such proteins might be able to be mobilised to assist with egg production without the need to break down the complex myofibrillar proteins that are responsible for muscle contraction. Houston *et al.* (1995a) showed that in the zebra finch there is a loss of both myofibrillar and sarcosplasmic proteins during egg formation, but among the sarcoplasmic proteins there is a heavy molecular weight protein which is preferentially lost, while levels of other sarcoplasmic proteins remain unaffected. This supports the hypothesis that bird muscle tissue contains sarcoplasmic proteins which can act as an amino acid reserve. It has already been demonstrated that a reduction in muscle condition can impair flight performance (Chapter 3), and may thus elevate predation risk. However, if females lay down sarcoplasmic storage proteins in their muscles prior to laying, this may incur costs. The carrying of extra mass in the muscle that does not contribute directly to the contractile power of the muscle may incur a penalty in flight performance analogous to the cost of carrying fat stores in winter (Witter & Cuthill 1993). This chapter investigates whether there is evidence to support this hypothesis. If female birds do store muscle proteins for use in egg production it would be predicted that prior to breeding females would possess more muscle tissue for their size than males. It would also be predicted that since some of this muscle tissue is comprised of sarcoplasmic storage reserves

which may not contribute directly to the contractile function of the muscle, there will be a cost in the carrying of this additional tissue in the form of reduced flight performance.

In this study therefore, an attempt is made to determine whether there is any gross physical evidence that female zebra finches do store nutrients and whether this entails a cost in terms of a reduced ability to escape from predators. To do this, measures of male and female muscle condition prior to breeding are compared, and how this affects alarmed flight performance at take-off is investigated.

Methods

A total of 17 female zebra finches, and 15 males were maintained in single sex pairs on an artificial 14L:10D light regime, with a diet of surplus ad libitum mixed seed and free access to water, grit and cuttlefishbone. Flight performance was assessed by recording the time taken to complete vertical flights when alarmed, since alarmed flights are a better indicator of predation risk than unalarmed flights (Veasey *et al.* 1998). Flights were conducted in a vertical flight aviary (190 × 100 × 100 cm). On each occasion that a bird was flown, it was taken from its home cage, placed in a holding chamber and weighed using a balance accurate to 0.1g. The holding chamber containing the bird was then placed in a sheath at the base of the flight aviary. The sliding lid of the holding chamber was transparent, allowing the bird to observe the nature of the flight it was required to make prior to its release. The lid of the holding chamber was then pulled open, and given a tap to startle the bird into making a vertical escape flight up to the perch at the top of the aviary. Vertical baffles (40 cm high and 20 cm apart) were positioned either side of the sheath in order to make the birds fly in as vertical a trajectory as possible. The baffle facing the observer was transparent, and the far baffle was opaque and marked at heights of 15 and 30 cm from the top of the release cup (25 and 40 cm from the ground respectively) such that the time taken to reach these points from the top of the release cup (i.e. when the bird was visible) could be calculated.

The bird was then re-caught with a net, returned to the holding cup and allowed to recover for a minimum of 30 s before being made to repeat the flight. Each daily flight test consisted of recording three vertical flights, from which a mean daily flight performance was calculated for the two distances. Flights were filmed using a camcorder, and the resulting video film was played through a digital video timer. This was then re-recorded on a VCR so that digital time measures accurate to 0.01s were encoded on to each frame. This film was then played back on the VCR using a frame by frame facility to measure the time taken for each bird to complete the defined section of each flight trial.

Each bird was flown on 15 separate days, with each trial comprising of three flights. From these a mean flight performance for each bird was calculated prior to pairing with males, i.e. when in the pre-laying condition. In addition to this, I collected immediate post-laying data (i.e. before females could replenish protein stores after having completed a clutch) from 17 other females in the form of three flight measures together with measures of muscle condition index which were compared with pre-breeding female data and with data derived from males.

Pectoral muscle condition was estimated using a moulding technique modified from Selman & Houston (1996b). Individual birds were placed breast downwards into a tray of dental alginate (Cavex CA37 Superior Pink), which gives a faithful mould without adhering to feathers (Selman & Houston 1996). The mould was then cut with a blade dorso-ventrally at the mid-point between the fulcra and the posterior portion of the sternum. Ten prints were then taken using an ink pad from the cut surfaces of the mould (five from each half). A horizontal line was then drawn at a perpendicular distance of 5 mm from the keel of each print. This distance represents an approximation of the average keel depth in zebra finches, and the position of the prints (the mid point along the keel length) corresponds to where the keel would have been at its deepest. This enclosed cross-sectional area of the pectoral muscle was measured using a computer plotter (BBC Master computer with cherry digitiser and puck), and a mean taken of measures from five separate prints, at least two being from each

half of the mould. A test for repeatability (Lessells & Boag 1987) has shown the five measures of the mould to be repeatable, such that means of these measures can be used with confidence (see Chapter 3). The mean muscle cross-sectional area is hereafter termed the muscle condition index. Mean flight times were then regressed against mean muscle condition index for males, and for females before and after laying.

Results

An ANCOVA demonstrated that the pre-laying relationship between the covariate body mass, and the dependent variable muscle condition, differed between the sexes (Effect of sex: $F_{1,29}$=8.711, P=0.006; effect of body mass: $F_{1,29}$=3.608, P=0.067, see figure 1). Figure 1 clearly shows that for a given mass, females tend to have larger indices of muscle condition. There was however, no significant interaction between sex and body mass (effect of sex x body mass: $F_{1,28}$=0.037, P=0.850). Thus, although there was no significant difference in body mass between the sexes (t-test, $t_{19.15}$=2.06, P=0.054, mean mass of males = 16.49±0.59SE, mean mass of females = 15.18±0.24SE) prior to breeding, males possessed on average 10% less flight muscle tissue than females, and subsequently males had 16% lower flight muscle ratio (flight muscle mass/body mass) than females (t-test, t_{30}=-3.717, P=0.001, mean muscle ratio of males = 4.28±0.16SE, mean muscle ratio of females = 5.11±0.15SE). This supports the prediction that females carry a protein store in their flight muscles prior to breeding.

No significant differences were found in the pre-laying flight times between the sexes (15 cm vertical flight times: t-test, t_{30} = -0.254, P = 0.801, 30 cm vertical flight times: t-test, t_{30} = -0.976, P = 0.337). A multiple stepwise linear regression of mean flight times against mean body mass and muscle condition was calculated for both sexes. In females, pre-laying muscle condition alone had a significant effect on flight performance at take-off (15 cm vertical flight, effect of muscle condition index: $F_{1,15}$=11.852, P=0.004, adjusted R^2=0.404 [see figure 2]; effect of mass: P=0.077, 30 cm vertical flight, effect of muscle condition index: $F_{1,15}$=11.440, P=0.004, adjusted R^2=0.395, effect of mass:

P=0.094), with birds with larger indices of muscle condition flying slower than those possessing smaller indices. In males, neither muscle condition nor mass had a significant effect on mean flight performance (15 cm vertical flight, effect of muscle condition index: $F_{1,13}$=0.789, P=0.391, adjusted R^2=0.015 [see figure 3]; effect of body mass: $F_{1,14}$=0.144, P=0.710, adjusted R^2=0.065, 30 cm vertical flight, effect of muscle condition index: $F_{1,13}$=2.364, P=0.148, adjusted R^2=0.089, effect of body mass: $F_{1,13}$=1.912, P=0.190, adjusted R^2=0.061). The absence of a relationship between muscle condition index and flight performance in males (figure 3), is in stark contrast to the relationship seen in females prior to laying (figure 2).

Although the mean measures of muscle condition index were smaller in post-laying females (mean pre-laying female muscle condition index = 77.27±2.22SE, mean post-laying female muscle condition index = 73.84±1.51) these differences were not significant (t-test, $t_{28.152}$=1.278, P=0.212). However, this probably arose because the analyses were not paired as in other experiments, or because females had not relied unduly upon endogenous stores in this breeding attempt. A significant difference between pre and post-laying muscle condition has however been demonstrated before (Chapters 5 & 6, Houston *et al.* 1995a). Thus in this case, as figure 4 shows, post-laying females still had significantly greater muscle condition for their body mass than did non-breeding males (ANCOVA, effect of sex: $F_{1,29}$=4.538, P=0.042; effect of body mass (covariate): $F_{1,29}$=2.808, P=0.105, interaction between sex and body mass: $F_{1,28}$=1.758, P=0.196).

As figure 5 shows, no effect of muscle condition was found upon flight performance for post-laying females (15 cm vertical flight, linear regression, effect of post breeding muscle condition index: $F_{1,15}$=1.947, P=0.183, adjusted R^2=0.056, 30 cm vertical flight, linear regression, effect of post breeding muscle condition index: $F_{1,15}$=2.570, P=0.130, adjusted R^2=0.089).

Discussion

These results indicate that a real difference appears to exist prior to breeding between the flight muscles of male and female zebra finches, since females typically have a higher flight muscle ratio and muscle condition index than males. In neither males or females was a significant relationship between flight muscle ratio and flight times found. Moreover, in females, larger flight muscles appear to incur a cost in terms of reduced alarmed flight velocity, but not in males, contrary to the findings of Marden (1987) who found that animals with a greater flight muscle ratio were capable of generating more power at take-off. Chapter 2 clearly demonstrated that loss of muscle condition resulting from laying, was correlated with losses in maximal flight velocity. It is notable however, that slight losses in muscle condition caused slight improvements in flight performance, and only after approximately 4-7% of flight muscle condition was lost were reductions in flight velocity seen (see Chapter 2). It is possible that this initial loss in muscle tissue represents the storage component which can be lost without any impairment of flight performance. Muscle loss beyond that level might then represent losses of contractile components of flight muscle tissue, and hence produce reductions in flight performance. In this experiment, females were found to have higher levels of flight muscle tissue per gram of body mass than males. This extra muscle tissue might thus represent the storage component required for egg production, or alternatively provide extra power to enable the female to carry her eggs. The reduced flight performance in females possessing larger flight muscle condition indices supports the first interpretation, as does the finding that this relationship breaks down after a clutch is completed. Females thus appear to pay a cost prior to laying, in that the storage of proteins (and possibly other nutrients) impairs their flight performance.

In the past, when biologists have searched for costs associated with reproduction, they have typically focused upon the costs which occur during or after the breeding period such as reduced survival or fecundity as a result of attracting mates (Ryan 1985), lactation (Clutton-Brock *et al.* 1982), the establishment and defence of territories (see Huntingford & Turner 1987), nest

building (Gauthier *et al.* 1996), rearing young (Gustafsson & Sutherland 1988) possibly mediated through a reduction in immune responsiveness (Nordling *et al.* 1998) etc. In this experiment we have discovered a potential cost of breeding, which affects female birds prior to the onset of the reproductive period. Female zebra finches are opportunistic breeders dependent upon rainfall and green seed availability which is relatively unpredictable over time (Zann & Straw 1984, Alan & Hume 1997). It is therefore advantageous for females to be physiologically capable of rapidly initiating a breeding attempt so that they can make the most of these short and temporally unpredictable windows of opportunity. This is particularly so since the larger protein reserves available to a female, the larger and better quality clutch she is likely to lay (Houston *et al.* 1983). Moreover, for the same sized clutch she will lose relatively less muscle condition than a bird with poorer protein reserves, with its negative consequences on flight performance (see Chapter 6). However, the results from this experiment show that these benefits must be weighed against the costs associated with the possession of such stores in terms of reduced alarmed flight performance prior to laying, not to mention the costs associated with the acquisition of those nutrients.

Fig. 1.

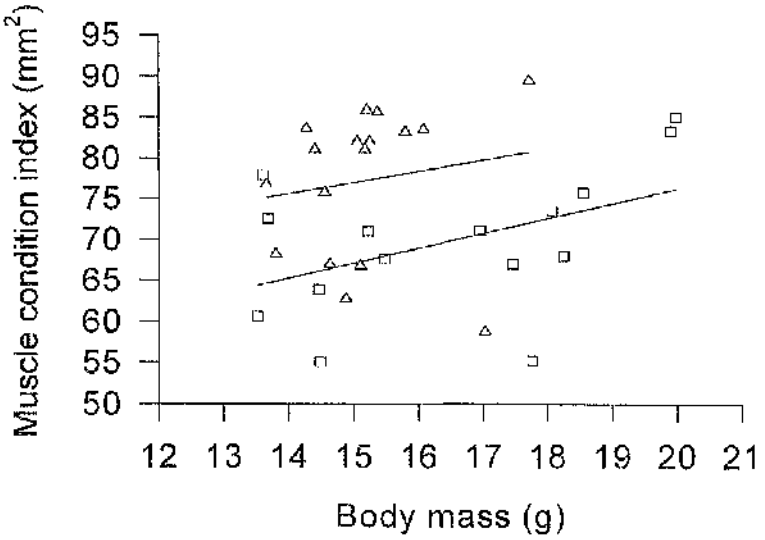


Fig. 2.

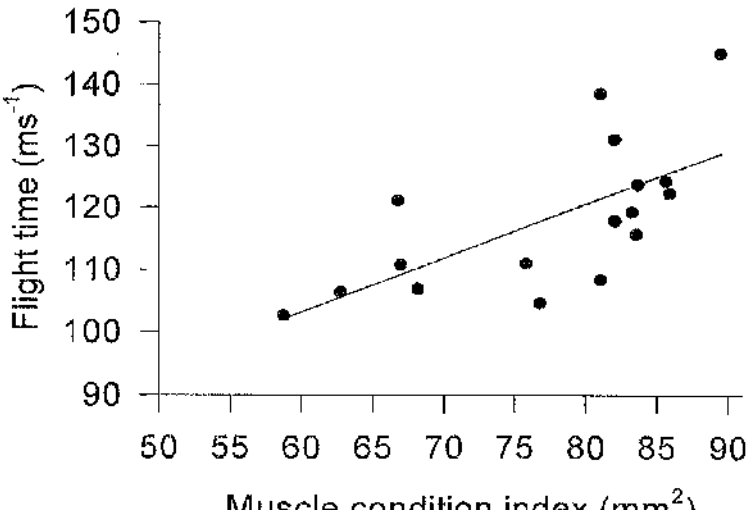


Fig. 1. The relationship between mean body mass and muscle condition index for male and pre-laying female zebra finches. Males = open squares, females = open triangles.

Fig. 2. The relationship between pre-laying muscle condition index and mean time taken to fly to a height of 15 cm for female zebra finches prior to laying.

Fig. 3.

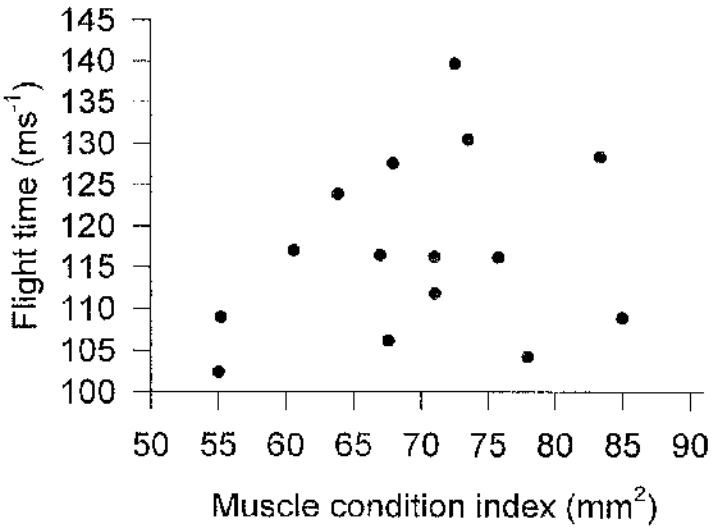


Fig. 4.

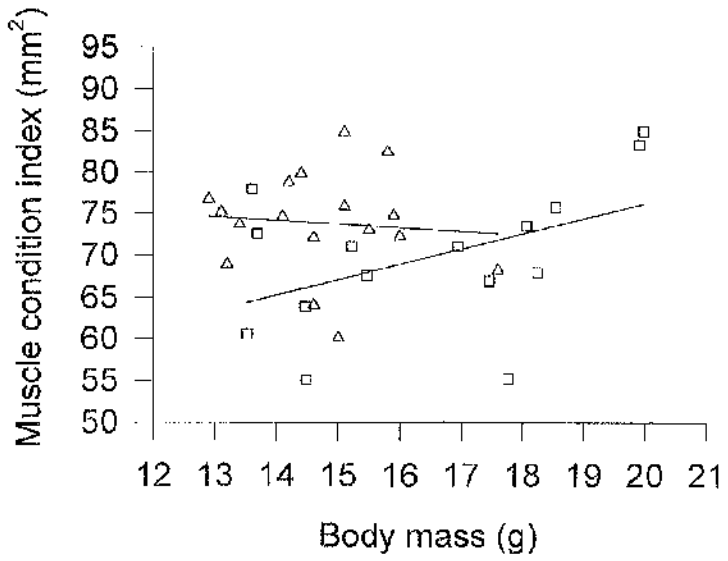


Fig. 3. The relationship between pre-laying muscle condition index and mean time taken to fly to a height of 15 cm for male zebra finches.

Fig. 4. The relationship between mean body mass and muscle condition index for male and post-laying female zebra finches. Males = open squares, females = open triangles.

Fig. 5.

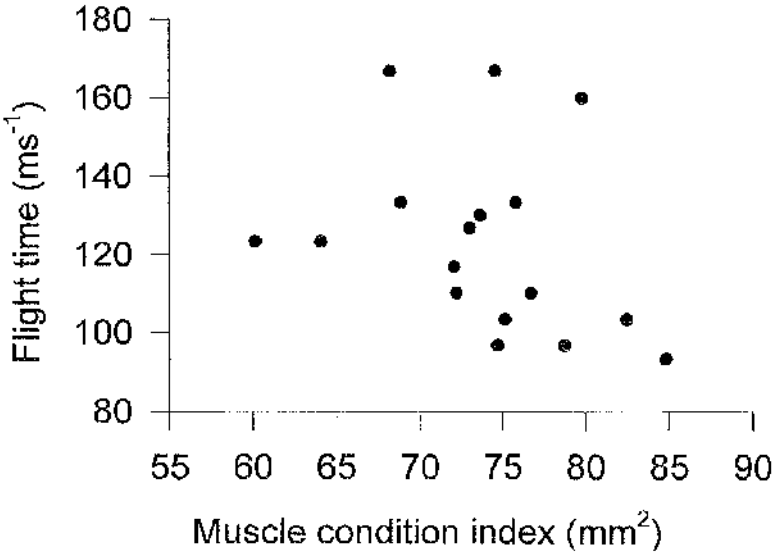


Fig. 5. The relationship between post-laying muscle condition index and mean time taken to fly to a height of 15 cm for female zebra finches after having completed a clutch.

Chapter 5

Clutch size, muscle condition and predation risk

Abstract

While the trade-off between reproduction and survival is a central tenet of life history theory (Stearns 1992) the underlying mechanisms are poorly understood (Tatar & Carey 1995). We demonstrate experimentally that when laying larger clutches female zebra finches (*Taeniopygia guttata*) lose more flight muscle condition and become significantly slower to make flights of the type made when attacked by predators. Moreover, individual birds show a trade-off between muscle tissue invested in reproduction and escape flight speed. This is the first demonstration of a physiological trade-off between resource allocation to reproduction and to the maintenance of musculature critical to an animal's escape response. The reduction in muscle condition during laying may thus constrain clutch sizes in birds vulnerable to predation.

Introduction

Egg production in birds has often been considered as a relatively inexpensive process, since they may readily replace lost eggs and even entire clutches (Monaghan & Nager 1997). This assumption has meant that in the majority of studies investigating reproductive costs in birds, egg production is ignored, and only the costs associated with incubation and rearing young are typically considered (Monaghan & Nager 1997). It is perhaps not surprising that as many as 30% of studies failed to find a cost of avian brood enlargement on either parents or offspring (Stearns 1992), when only 3.1% of these studies manipulated the number of eggs laid (Monaghan & Nager 1997). Since many bird species utilise proteins from their own muscles in the formation of eggs (Houston *et al.* 1995a), it is to be expected that larger clutches will cause greater declines in muscle condition (Monaghan *et al.* 1998). The ratio of flight muscle mass to body mass has been shown to affect take-off capability across a variety of taxa (Marden 1987). Therefore any reduction in the mass of the flight muscles due to increased reproductive effort might be expected to reduce the

ability to take flight rapidly, as when escaping predators. Previous studies on the effect of reproduction on predation risk have, however, typically concentrated upon the increased likelihood of a predatory attack, rather than an increased risk of capture once an attack has been initiated (Magnhagen 1991, Székely *et al.* 1994). Here we test for the first time whether there is a trade-off between a bird's clutch size and its ability to escape rapidly from predators, mediated through a reduction in flight muscle size.

Materials and Methods

Two treatments were utilised in which the same established pairs of zebra finches were manipulated into laying either large or small clutches in successive breeding attempts. In small clutch treatments, artificial eggs were added to the nest boxes daily for four days, starting on the fourth day after pairing, thus inducing the laying of a smaller clutch (Haywood 1993ab); in large clutch treatments, the first four eggs were removed on the day each was laid in order to induce the laying of a larger clutch (Haywood 1993ab). To synchronise laying, the sexes were kept apart until required to start breeding, at which point pairs were reunited, provided with nest building material and nest boxes. Upon completion of a clutch (i.e. when no further eggs had been laid for two days), the pairs were separated, nestboxes removed, and the birds left to recover for between 4-6 weeks before the next breeding trial. Trials alternated between large and small clutch treatments.

Flight performance was measured in a flight aviary (100 cm x 100 cm x 190 cm high) separate from the cages in which the birds were housed. Females were trained to fly vertically during the period prior to pairing until they showed no further improvement in performance with time, and were then assessed for flight performance every day on which they laid an egg after pairing. On each occasion that a bird was flown, it was removed from its home cage, placed in a holding chamber and weighed to the nearest 0.1 g. The chamber was then placed in a sheath at the base of the flight aviary, from where the bird was released and immediately induced to make a near vertical escape flight to a perch at the top of the cage. The bird was then re-caught, returned to

the holding chamber and allowed to recover for a minimum of 30 s. Each trial consisted of three vertical flights after which the bird was returned to its breeding cage. Flights were filmed using a camcorder, and the mean time taken to reach 30 cm from the top of the holding chamber was calculated from analysis of the three recorded flights. Although body mass has only a minor effect, if any, upon alarmed flight velocity within the natural weight range of a number of bird species studied including the zebra finch (Witter *et al.* 1994, Lee *et al.* 1996, Kullberg *et al.* 1998, Kullberg 1998, Veasey *et al.* 1998, Lind *et al.* in press), all trials were conducted between three to five hours after dawn, when body mass is most stable in order to minimise any confounding effects due to the natural diurnal variation in body mass (Dall & Witter 1998).

An index of female flight muscle condition based on the maximum cross-sectional area of the pectoral muscles was determined at the time of pairing and after the completion of each clutch. Individual birds were placed breast downwards into a tray of dental alginate (Cavex CA37 Superior Pink), since this gives a faithful mould without adhering to feathers (Selman & Houston 1996b). The mould was then cut with a blade dorso-ventrally at the mid-point between the fulcra and the posterior portion of the sternum (i.e. where the keel is deepest). Ten prints were then taken from the cut surfaces of the mould (five from each half). A horizontal line was then drawn at a perpendicular distance of 5 mm from the position of the keel of each print, 5 mm being the average keel depth in zebra finches. The enclosed area was then measured using a computer plotter (BBC Master computer with cherry digitiser and puck), and a mean cross-sectional muscle area calculated from measures taken from five separate prints, at least two being from each half of the mould. This muscle area is defined as an index of pectoral muscle condition.

All females (n=18) completed at least two clutches under both large and small clutch regimes: in the following analyses we compare the mean change in flight performance and mean change in pectoral muscle condition of each female under the two treatments. We also calculate regression lines of changes in

muscle condition against change in flight performance for individual birds, using one data point per breeding attempt.

Results

As implicated, the manipulation of clutch sizes resulted in birds laying lighter clutches, containing fewer eggs, when on the small clutch treatment compared to the large clutch treatment (figure 1a). Females lost the same amount of body mass during egg laying, whether on the large or small clutch treatment (figure 1b). However, they lost substantially more pectoral muscle condition when induced to lay large clutches than when induced to lay small clutches (figure 1b). The changes in flight muscle condition were reflected in flight performance at the end of laying: females flew quicker after the small clutch treatment than after the large clutch treatment (figure 2a). There was also a significant effect of clutch treatment on the change in flight performance during laying: when induced to lay small clutches, birds flew faster at the end of laying than at the beginning, whereas when induced to lay large clutches the same birds flew slower after completing the clutch, despite having lost weight during laying (figure 2b).

Individual trade-offs of the change in escape flight ability against the change in muscle condition during laying were calculated for those females that showed a minimum of 15% variation in the changes in muscle condition index in different breeding attempts. Twelve of the 14 females in the analysis showed a negative relationship between change in muscle index and change in flight performance, a significant departure from the null hypothesis that relationships were equally as likely to be positive as negative (Binomial test, N=14, P = 0.013, figure 3).

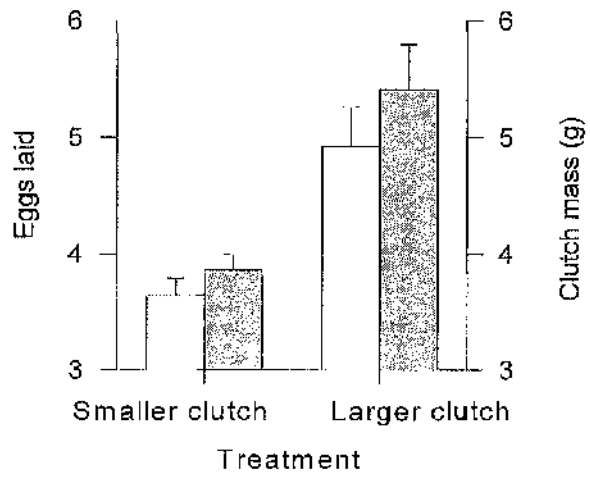
Discussion

Our results show for the first time that increased egg production entails a within-clutch cost in terms of decreased escape flight performance due to flight muscle loss. Previous studies on the effects of reproduction on locomotor performance in birds (Lee *et al.* 1996), reptiles (Schwarzkopf & Shine 1992)

and crustaceans (Berglund & Rosenqvist 1986) have concentrated on the costs of carrying eggs. In contrast, the reduction in escape ability measured here occurred after all the eggs in a clutch had been laid, and was independent of body mass, and must therefore be due to physiological changes associated with the production rather than the carriage of eggs. The observed decrease in escape flight speed may have significant survival costs, as the ability of small ground feeding birds to get airborne rapidly is the single most important factor determining their chances of escape when attacked by mammalian or avian predators (Rudebeck 1950, Page & Whitacre 1975, Cresswell 1993). In fact, it has been suggested that predation risk per attack increases exponentially with time to reach protective cover (Bednekoff 1996). A comparison of the two clutch treatments showed that females flew approximately 7% slower when laying an average of 1.3 extra eggs per clutch. This difference in maximal escape speed may have significant survival implications when under attack from raptors, since speeds of up to 100 ms^{-1} have been recorded for the peregrine (*Falco peregrinus*) based on timed flights over known distances (Hantge 1968), or up to 58 ms^{-1} for radar measurements of a domesticated gyrfalcon (*Falco rusticolus*) (Tucker *et al.* 1998). These speeds will result in the predators travelling an additional 70-120 cm towards the finch in the extra time that it would take to reach a height of 30 cm from the ground having laid a larger clutch. It can thus be seen, that by slightly increasing her clutch size, a zebra finch may severely jeopardise her survival from a predatory attack, and thus her entire residual reproductive value. The demonstrated trade-off between reproductive investment and subsequent impairment of escape ability within individuals, may thus be sufficient to constrain clutch sizes in birds vulnerable to predation.

Fig. 1.

a.



b.

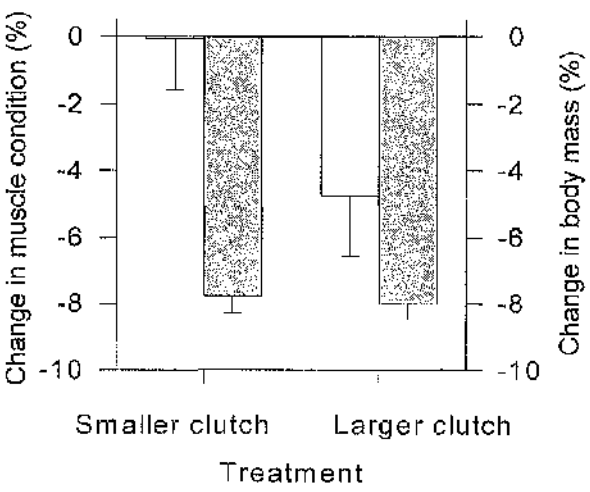
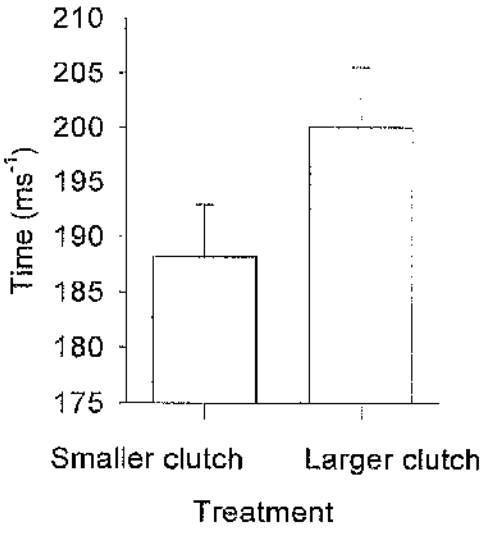


Fig. 1. Mean (±SE) reproductive parameters of 18 females when subjected to the two contrasting clutch manipulations. The smaller clutch treatment resulted in (a) fewer eggs being laid (open bars, paired t-test: t_{17}=3.88, P=0.001), with a lighter overall clutch mass (shaded bars, paired t-test: t_{17}=4.41, P=<0.001) than the large clutch treatment, and (b) in smaller declines in muscle condition (open bars) than the large clutch treatment (Wilcoxon matched pairs test: Z=-2.55, N =18, P=0.011), though the treatment did not affect the loss of body mass during laying (shaded bars, Wilcoxon matched pairs test: Z=-0.59, N =18, P=0.557).

Fig. 2.

a.



b.

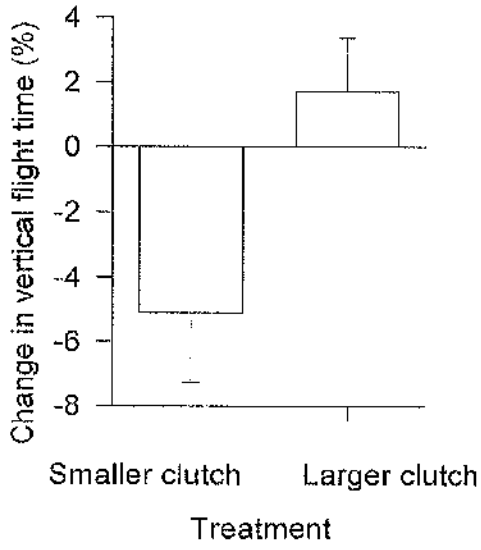


Fig. 2. The effect of clutch treatments upon mean (±SE) initial escape flight performance. (a) When induced to lay larger clutches, birds were slower to reach a height of 30 cm (paired t-test: t_{17}=2.27, P=0.037). (b) The effect of treatment upon the change in escape flight time between the first and last egg of a clutch. Flight times decreased during egg laying in the small clutch treatment, and increased in the large clutch treatment (Wilcoxon matched pairs test: Z=-2.069, N =18, P=0.039).

Fig. 3.

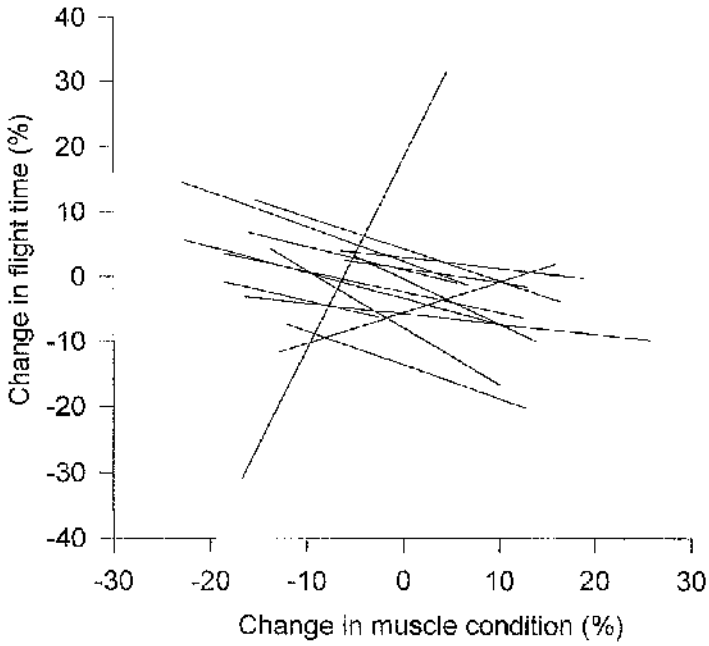


Fig. 3. The trade-off for individual birds between changes in muscle condition index during egg laying and change in escape flight time. Shown are the regression lines for 14 females, each based on a minimum of four breeding attempts per bird, with one data point per clutch.

Chapter 6

State dependent reproductive costs: The effect of pre-breeding diet on subsequent predator avoidance

Abstract

It is widely accepted that reproduction is costly, but the underlying mechanisms are often poorly understood since costs may be paid long after the period of investment. Here the demands of egg production in female birds is investigated in terms of impaired ability to escape from predators *after* the completion of a clutch. It is shown that egg production, through its depletion of flight muscle proteins which are used to provision developing eggs, causes a reduction in maximal flight performance. Furthermore, it is shown that this effect varies with the nutritional state of the female prior to breeding. By experimentally manipulating the pre-breeding diet of zebra finches it is shown that when females feed on high quality protein foods prior to breeding they were faster to make escape flights after egg-laying, whereas when the same birds were only able to feed on lower quality protein food they were slower. This difference was mediated through differential shrinkage of the flight musculature, and was independent of clutch size, clutch mass or the females' body mass. This is the first demonstration of an effect of diet quality on predator evasion, and illustrates state-dependent reproductive costs independent of offspring number.

Introduction

Both life history theory and empirical evidence suggest that current reproduction incurs costs likely to diminish the prospects for reproduction in the future (reviewed by Partridge & Harvey 1988, Roff 1992, Stearns 1992). The decrease in residual reproductive value arises through a reduction in either survival or future fecundity. However, the mechanisms underlying these trade-offs are rarely understood, as it is often difficult to measure how current reproduction can alter parental survival probabilities or future reproductive capacity. One candidate mechanism for a decrease in survival rate in reproducing animals is an elevated risk of predation. In this study, the potential

effect of egg production upon the ability of female birds to escape from a predator is examined; the novelty of the approach is firstly that an impairment of escape performance *after* the eggs have been laid is demonstrated (so that it is due to the physiological demands of producing eggs, and not simply a consequence of carrying them [Jones 1987, Lee *et al.* 1996]), and secondly that this cost of reproduction is dependent on the pre-breeding nutritional state of the female.

The acquisition of nutrients for egg production is a costly process for most birds. While species vary widely in the extent to which they derive these nutrients from current food intake or from body reserves (Drent & Daan 1980), Houston *et al.* (1995a) found that 21 out of 29 species exhibited a significant loss of muscle mass at the time of laying, suggesting that the use of endogenous reserves is widespread. The amino acid balance of egg proteins is unusual, having particularly high levels of certain amino acids which can be limiting in the natural food supply of a bird (Ramsay & Houston 1997). Since it has been demonstrated that birds use muscle proteins as a source of amino acids for egg production (Houston *et al.* 1995bc), females with high quality endogenous protein stores may be better able to supply limiting amino acids for egg production than birds with poor quality muscle stores. Evidence in support of this comes from Selman & Houston (1996a), who showed that the extent of muscle mass lost by zebra finches (*Taeniopyga guttata*) during egg laying was strongly influenced by the quality of diets available before the start of laying, with females given a high protein supplement prior to laying losing less muscle mass per gram of egg laid than those maintained on a low protein diet. Since both groups of birds had similar muscle mass at the start of laying and were on identical diets during the period of egg production, these effects must have been mediated through differences in the condition of their muscles prior to laying. Selman & Houston (1996a) suggested that birds with access to high quality protein prior to breeding were able to develop protein stores with the optimum amino acid balance for egg production, and subsequently only needed to break down comparatively small quantities of muscle protein to provide the required amino acid balance for egg production. Birds maintained on a poorer

nutritional diet might lack adequate specialised protein stores, and so might need to break down larger quantities of general muscle proteins to provide the nutrients for egg formation. These findings have obvious implications for the flying abilities of the female.

Flight performance at take-off is likely to influence the risk of being caught by a predator (Rudebeck 1950, Page & Whitacre 1975, Cresswell 1993, Bednekoff 1996). Therefore reproduction may directly reduce survival through the effect that egg production has on the musculature needed to escape from an attacking predator. Moreover, the extent of this cost of reproduction should be dependent on the muscle condition of the female at the time of laying, and hence her pre-laying diet. Here we test this prediction in an experiment that manipulated the same birds into producing the same clutch size in successive breeding attempts, but after having received different pre-laying diets. The results demonstrate a mechanism behind a state-dependent cost of reproduction: a poorer pre-laying diet resulted in females breaking down more of their flight muscles to produce eggs, so causing a reduction in their ability to escape rapidly from predators after the eggs were laid.

Methods

Pairs of zebra finches were kept in breeding cages (60 x 50 x 40 cm) under a regime of 16 hours of light per day. In order to synchronise laying, sexes were kept apart until the breeding portion of each trial, at which point pairs were reunited and cages were provided with nest building material and nest boxes. Two treatments were utilised in order to manipulate the costs of producing a particular clutch size. In the low quality diet (LQD) treatment birds were maintained throughout a breeding trial on a diet consisting of mixed seed. In the high quality diet (HQD) treatment, females were given a supplement of homogenised boiled hens eggs in a rusk base which has been shown to reduce muscle loss at the time of laying (see Selman & Houston 1996a), in addition to the mixed seed diet, for four weeks prior to pairs being reunited (at which point the supplement was stopped). From the time of pairing and throughout the period of egg formation and egg laying both groups were therefore on identical

seed diets, and previous studies showed that daily seed consumption between the two groups did not differ (Selman & Houston 1996a). In all treatments, birds were given free access to water, grit and cuttlefishbone. In order to encourage females to lay same sized clutches, we compensated for the known effect that these diets normally have upon clutch size (Selman & Houston 1996a) by manipulating the female into laying more or fewer eggs (Haywood 1993ab). Thus when birds were on the LQD treatments, we removed the first four eggs of a clutch on the day each was laid in order to induce the laying of a larger clutch than might otherwise have been laid. Conversely, when birds were on the HQD treatment, a single artificial egg was added to the nest box on the first four days after pairing in order to induce the laying of a smaller clutch than the female would normally lay given her nutritional condition (Haywood 1993ab). Eggs were weighed on the day of laying. Once the clutch was completed (i.e. no new eggs having been laid for two days), the members of a pair were placed in separate cages without nestboxes and left for six weeks to allow them to recover. They were maintained on the appropriate pre-breeding diet for the next trial for the last four of the six weeks.

Flight performance was assessed by video recording alarmed vertical flights since they are better indicators of changes in predation risk than unalarmed routine flights (Veasey *et al.* 1998). Females were given regular experience of the flight protocol during the recovery period between breeding attempts, to ensure that they were accustomed to the flight protocol (see Veasey *et al.* 1998 for further details). Once pairs had been re-established, females were flown only on the days on which they laid an egg. On each occasion that a bird was flown, it was taken from its home cage, placed in a holding container and weighed to the nearest 0.1g. The holding container was then placed in a sheath at the base of a vertical flight aviary, from where the bird was startled into making a escape flight 1.25 m up to a perch at the top of the aviary. The bird was then re-caught, returned to the holding container and allowed to recover for a minimum of 30 s before being flown again. A total of three flights were made on each day that a female laid an egg. The mean time taken to reach 30 cm from the top of the release cup was calculated from an analysis of the video

footage. Although body mass has been shown to have a minimal effect on alarmed flight velocity (Witter *et al.* 1994, Lee *et al.* 1996, Kullberg *et al.* 1998, Kullberg 1998, Veasey *et al.* 1998, Lind *et al.* in press), in order to minimise any confounding effects which might occur as a result of the diurnal variation in body mass in zebra finches (Metcalfe & Ure 1995, Dall & Witter 1998), females were flown at a time when their mass was most stable, between three to five hours after dawn (Dall & Witter 1998).

To investigate changes in female pectoral muscle condition associated with reproduction, an index of muscle condition was calculated by recording the profile of the pectoral muscles of females using a technique modified from Selman & Houston (1996b). The pectoral region of live birds was moulded in dental alginate (Cavex CA37 Superior Pink), which gives a faithful mould but does not adhere to feathers. The mould was then cut with a blade dorso-ventrally at the mid-point between the fulcra and the posterior portion of the sternum. The cut surfaces of both halves of the mould were then inked, and five prints were taken of each half. A mean measure of the maximal cross sectional area up to 5 mm from the base of the sternum was then calculated from five of the prints using a computer plotter (BBC Master computer with cherry digitiser and puck). An analysis of variance for the calculation of repeatability (Lessells & Boag 1987) performed across the five measurements confirmed that the repeatability was high ($F_{19,99}$=17.825, r = 0.771). The mean cross sectional pectoral muscle area is hereafter referred to as the muscle condition index.

The breeding trials alternated between HQD and LQD treatments, and females were only included in the analyses if they had laid at least one clutch under both treatments. In all, 19 birds completed at least one breeding trial under both treatments, and 14 of these were also measured for pre and post-laying muscle condition under both treatments. All analyses are in the form of paired comparisons of the mean laying and flight performance of the same bird under the two treatments.

Results

No differences were found in clutch masses (paired t-test: t_{18}=0.65, P=0.523), nor in the number of eggs laid (paired t-test: t_{18}=1.25, P=0.234, figure 1) between the two diet treatments, indicating that the egg manipulations had successfully standardised the clutch size for the two treatments. The treatments did not produce any differences in the female's body mass after laying her first egg (paired t-test: t_{18}=1.73, P=0.100) or last egg (paired t-test: t_{18}=1.53, P=0.143), nor in the percentage change in body mass between first and last eggs (Wilcoxon matched pairs signed-ranks test: Z=-0.242, N =19, P=0.809, see figure 2). However, while no differences were found in the muscle condition index prior to breeding (paired t-test: t_{13}=-1.57, P=0.140), the percentage change in muscle condition during the laying of a clutch was significantly different between the two treatments (Wilcoxon matched pairs signed-ranks test: Z=-2.794, N=14, P=0.005), with the LQD treatment causing birds to lose muscle condition during laying whereas the muscle condition of the same birds slightly improved after laying a clutch on the HQD treatment, despite the same decline in body mass (figure 2).

The time taken by females to reach a height of 30 cm at take-off was not significantly different between the two treatments on the day the first egg was laid (paired t-test: t_{18}=-1.00, P=0.329). However, by the end of laying a clutch, birds were significantly slower when on the LQD than when on the HQD treatment (paired t-test: t_{18}=2.35, P=0.031, figure 3). The percentage change in flight performance (time to reach 30 cm) between first and last egg was significantly different in the two treatments (Wilcoxon matched pairs signed-ranks test: Z=-2.052, N=19, P=0.040, figure 4) with the LQD treatment causing birds to fly slower towards the end of a clutch, whilst when on the HQD treatment, the same birds tended to fly faster as the clutch was completed. A similar result was obtained when comparing the mean change in flight time between the pre-breeding period and the day of laying the last egg (Wilcoxon matched pairs signed-ranks test: Z=-1.972, N =19, P=0.049).

Discussion

It is well known that animals tend not to reproduce if their condition or nutritional intake is poor, due to an inability to provide sufficient resources both for themselves and for their young. The major constraint has usually been assumed to be energy. However, we have shown here that protein reserves may be of considerable importance. Our findings also suggest that in zebra finches it is the physiological process of producing eggs that is the cause of reduced flight performance in gravid birds, rather than the increase in mass associated with carrying them as has previously been suggested (Jones 1987). Firstly, the treatments caused differential changes in flight performance during laying without corresponding differences in body mass. Secondly, the changes in flight performance were dependent upon pre-breeding diet quality and not clutch size or mass, which were similar in the two treatments. Thirdly, the greatest reduction in flight performance was seen after the eggs had been laid. Moreover, body mass has a negligible effect on speed of escape flights in zebra finches (Veasey *et al.* 1998), unlike its effects upon routine flights (Metcalfe & Ure 1995).

When searching for a cost of reproduction, studies have in the past primarily considered the chick rearing phase in birds since egg production was considered to be relatively inexpensive (Lack 1947). However, an increasing number of studies have demonstrated that the energetic and nutritional costs of producing eggs are far from insignificant (Monaghan & Nager 1997), and even a modest increase in the number of eggs laid can cause significant reductions in the size of flight muscles (Monaghan *et al.* 1998) or chick provisioning rates, growth and survival (Heaney & Monaghan 1995). Muscle wastage may therefore form the basis of a physiological trade-off between reproduction and predation risk in birds, contributing to the determination of the optimal clutch size. Since the trade-off between reproduction and predation appears to be asymmetrical, with marginal increases in reproductive output jeopardising maternal survival and subsequently the female's entire residual reproductive output, there is potentially a strong selective pressure to depress clutch sizes

particularly in young birds of long lived species for whom future reproduction is a larger component of expected life time reproductive output.

Mountford's cliff edge hypothesis has been used to explain why mean clutches sizes are smaller than the most productive, since larger clutches 'fall off the cliff' relative to the survivorship of smaller clutches in poor years (Boyce & Perrins 1987). Morris (1996) demonstrated that this cliff edge effect may be state dependent in white footed mice (*Peromyscus leucopus*), as smaller mothers showed lower recruitment levels for large litters in comparison to larger mothers producing litters of the same size. Our current findings suggest that life history strategies may also be state dependent (see McNamara & Houston 1996) in the zebra finch, but in this case it is the survivorship of the female (and only indirectly that of the brood) that is the key. It is conceivable therefore that the extent to which clutch size is depressed below the maximum number of eggs the parents are capable of producing and rearing successfully may depend on this flight performance trade-off. Therefore the observed smaller clutch sizes of females on lower quality diets or in poorer condition (Selman & Houston 1996a) may not simply reflect their reduced ability to lay eggs, but may reflect a state dependent trade-off whereby birds in poorer nutritional condition depress reproductive output in order to maintain their flight capability and thus maximising their probability of surviving to breed again.

It has already been demonstrated that zebra finches given high protein pre-breeding supplementation usually lay heavier eggs and clutches (Selman & Houston 1996a, Monaghan *et al.* 1996), but it has now been shown that if encouraged to lay the same number of eggs, high protein supplemented birds, tend to lay heavier individual eggs (paired t-test: t_{18}=2.24, P=0.038), with birds on LQD laying eggs on average 14% lighter than when on HQD (see also Selman & Houston 1996a). Since various parameters of offspring fitness may correlate with egg size (Hill 1993, Amundsen 1996, Smith & Bruum 1998), our results confirm that pre-breeding diet quality may influence not only the cost of reproduction to the parent, but also the number and quality of offspring. Given

the importance of female nutritional condition in influencing maternal and offspring survival, it is perhaps not surprising that females will actively change their diet when coming into breeding condition or breed only when adequate resources are available (Jones & Ward 1968, Ankney & Scott 1980, Ankney & Afton 1988, Zann *et al.* 1995). It is also noteworthy that male zebra finches have been shown to have an active preference for females that have high quality protein reserves (Monaghan *et al.* 1996), since they are not only more likely to lay more and heavier eggs (Selman & Houston 1996a) but are also more able to survive a predatory attack during the breeding period.

Fig. 1.

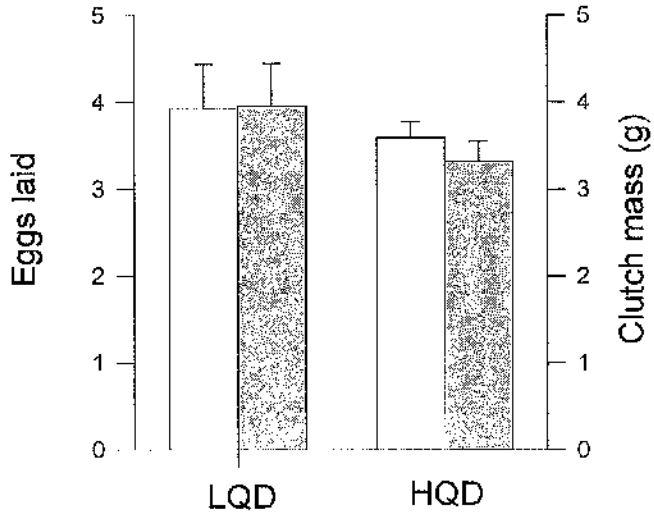


Fig. 2.

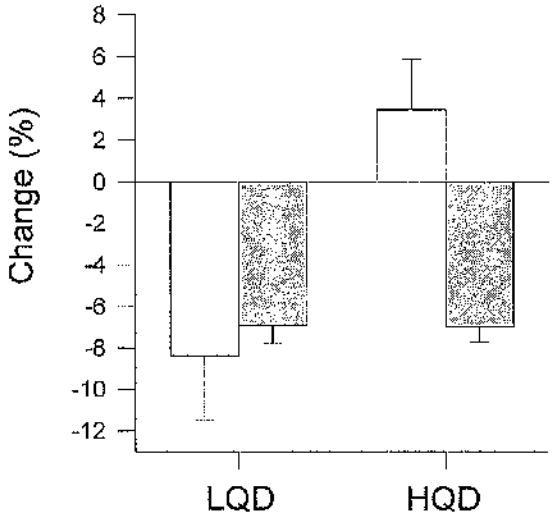


Fig. 1. The effect of treatment upon the mean (±SE) number of eggs laid (open bars) and mean (±SE) clutch mass (shaded bars). LQD = low quality diet, HQD = high quality diet.

Fig. 2. The effect of treatment upon the mean (±SE) change in muscle condition index (open bars) and mean change in mass (±SE) (shaded bars).

Fig. 3.

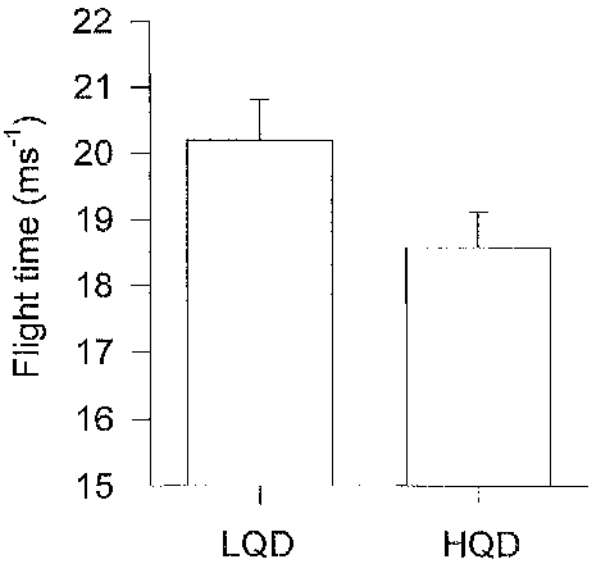


Fig. 4.

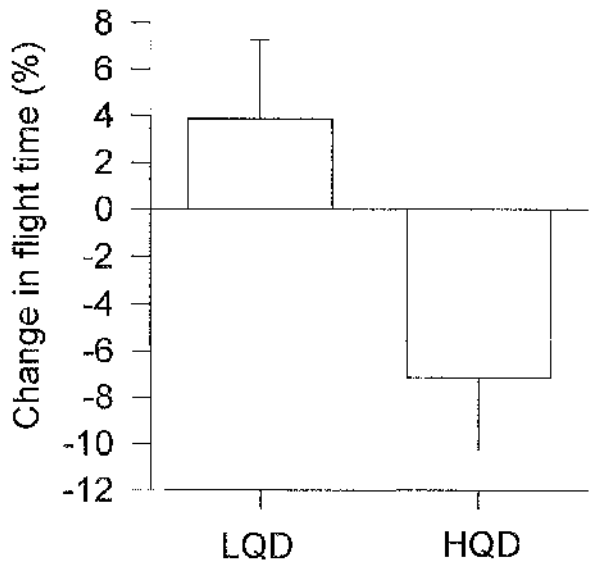


Fig. 3. The effect of treatment upon the mean (+SE) time taken to fly to a height of 30 cm at the end of laying a clutch.

Fig. 4. The effect of treatment upon the mean (±SE) change in the time taken to fly to a height of 30 cm between laying the first and the last egg of a clutch.

Chapter 7

Behavioural decisions made by laying female zebra finches (*Taeniopygia guttata*) under an elevated risk of predation

Abstract

It has long been known that reproduction may be costly. More recently the importance of predation risk as a cost of reproduction in females has begun to be appreciated. Since there may be a trade-off between reproduction and avoiding predation, one would expect animals facing such a trade-off to optimise this relationship. This may occur on an evolutionary level involving an adaptive change in the genome of a species over time, or through animals altering their behaviour, reproductive strategy or physiology in response to more short term changes in predation risk. Here we investigate whether breeding female zebra finches modify their behaviour to minimise their likelihood of being predated at the time of laying. In the first experiment, females were shown to alter their behaviour during laying in such a way as might minimise their predation risk: they fed less often, avoided high risk feeding patches, tended to associate with larger flocks, and spent more time in cover. However, the relationship between the male and female of a pair changed in such a way that would appear to elevate the female's risk from predation, with an increase in solo flights made by the female, a reduction in time spent with the male and in feeds made alongside the male during the laying period. It is likely that this is a result of the male's role in incubation forcing the pair to spend more time apart. In a second experiment, females deprived of cover were shown to feed more often with other birds, and feed less often and for a shorter duration in high risk areas than females provided with cover. These effects were especially pronounced during the egg laying period. Female zebra finches were therefore shown to be sensitive to changes in their predation risk, and demonstrated changes in behaviour likely to minimise any increases in predation risk whether due to environmental variables such as differences in cover or physiological variables such as the muscle loss which may result from laying eggs.

Introduction

An increase in maternal predation risk as a result of reproduction is a potentially widespread phenomenon in animals since changes in female condition during this time are likely to affect her escape capabilities. Slagsvold & Dale (1996) have demonstrated for example that predation rates of female pied flycatchers (*Ficedula hypoleuca*) are highest during the egg laying phase, whilst Berglund & Rosenqvist (1989) have shown that gravid prawns (*Palaemon adspersus*) are more conspicuous to predators, and Madsen (1987) has shown that the grass snake (*Natrix natrix*) suffers from decreased locomotor capabilities when gravid and is subsequently more vulnerable to predation. Animals might adapt to such increases in predation risk by undergoing inter-generational evolutionary changes, and/or by making intra-generational behavioural or physiological adaptations. Evolutionary changes might include a change in reproductive strategies whereby animals synchronise their reproduction to reduce the likelihood of predation on offspring or adults, evolve habitat preferences that reduce their exposure to predators, or possibly reduce their reproductive output. However, predation risk may be relatively invariant over evolutionary time, but may vary considerably on a seasonal, daily and even a minute by minute basis (Lima & Dill 1989). Consequently, animals must be able to assess such changes in predation risk, and make short term intra-generational adaptations to avoid predation. Such adaptations have been demonstrated in the southern water skink (*Eulamprus tympanum*) which when gravid adopts an anti-predator strategy that is more reliant upon crypsis due to its reduced mobility at this time (Schwarzkopf & Shine 1992).

It has already been shown that declines in pectoral muscle condition during laying result in a reduction in alarmed flight performance and subsequently an increased likelihood of predation (see Chapter 3). Moreover it has been found that when encouraged to lay a larger clutch, birds lost more pectoral muscle condition index and subsequently flew slower at the end of laying (see Chapter 5). In the following experiments, whether or not laying females adjust their behaviour to minimise any increase in predation risk which might result from

egg laying is investigated, and whether any such behavioural change varies with the riskiness of the habitat. In order to do this, two experiments were carried out. In the first experiment female zebra finches were given opportunities to adjust their perceived risk from predation by varying the extent to which they spent time flying and feeding, fed in high risk patches, sought cover, or flocked with bengalese finches (*Lonchura striata*) or the male zebra finch to which they were paired. In the second experiment, pairs of breeding zebra finches were again housed with bengalese finches, but the riskiness of the habitat was varied by altering the level of protective cover. From previous findings on flight performance during laying (see Chapter 3), one would expect female zebra finches to minimise their predation risk by reducing the times and extent to which they are vulnerable to predators during laying, and to do this to a greater extent if denied access to cover (Beck & Watts 1998). However, egg production is costly for female birds, both in terms of energy and nutrients (Houston *et al.* 1995a), consequently, there is likely to be a conflict between the need to minimise predation risks, and the need to satisfy the demands of egg laying. This may be particularly true in the light of experiments which showed that birds flew slower and lost more muscle condition if encouraged to lay when on a poorer diet (see Chapter 6). However, since failure to survive from a predatory attack will have a greater fitness cost than a reduction in foraging behaviour, it is to be expected that birds will modify their behaviour to optimise the trade-off between foraging and predation risk. Consequently, birds denied cover are likely to take fewer risks than those with cover, since their nutritional demands are likely to be the same, but their overall risk of predation is higher.

Experiment 1

Methods

A single pair of zebra finches was housed with eight male bengalese finches in four replicate outdoor aviaries measuring 6 m long by 4 m wide and 2.5 m high. The two species are related and will feed in mixed flocks, without the complications of sexual interactions between them. Consequently, we can assume that the primary reason a female zebra finch would choose to flock

with bengalese finches is due to the enhanced predator detection and avoidance that this would entail in accordance with the selfish herd theory (Hamilton 1971) and the many-eyes affect (Kenward 1978).

The aviaries were constructed of a wooden frame with wire mesh on all panels including the roof, with the exception of one of the 4 m by 2.5 m end panels which was of solid wood. Each aviary was divided along its length into six zones, one metre wide, enabling the position and subsequent associations between the birds to be recorded. The aviaries were furnished with composted wood bark flooring throughout, and numerous perches placed in the first 2 m of the cage from the solid end panel. Cover was also provided in this area in the form of military camouflage netting suspended from the roof (see figure 1). This area was considered to be equivalent to shrub or tree cover in the wild, since it had 3-dimensional complexity such that birds could hide in it, and represented a place of refuge above the ground. All birds demonstrated a strong preference for this area, spending a large proportion of their time perching there including roosting, and flying back there when disturbed.

Food was provided at two feeding sites placed in the fourth and sixth meter zones. Each feeding site had two food patches designed to create a low and high predation risk patch. The low risk patch comprised of a tray at ground level on which 15 g of mixed finch seed was placed daily. The high risk food patch consisted of a similar tray, containing the same amount of food, however, around this tray was placed a clear perspex tube, 25 cm high and 30 cm in diameter. This forced birds to take-off from the feeding tray in a near vertical trajectory and thus reduced the speed of escape flights when leaving the high risk patch, since birds face a trade-off between flight velocity and angle of ascent (Witter & Cuthill 1993). Video-recordings of the initial 150 cm section of alarmed flights made by zebra finches flying to cover from the two feeding patches, confirmed that flights were significantly slower from the high risk feeding patches than from the low risk feeding patches (T-test, N= 75, $t_{23.65}$=-6.59, P=<0.0001, figure 2). The greater time and manoeuvrability required to reach cover would therefore increase the perception of risk at this site. In order

to encourage birds to use the high risk feeding patch, seed in the low risk patches was mixed with 200 g of coral sand of a similar grain size to the seed, thus making feeding more time consuming. Moreover, since just enough food was provided in the four feeding patches to ensure that the majority of food was used up by the end of the day, birds were encouraged to feed in the high risk sites as food was depleted in the low risk sites. The birds thus faced a choice of feeding between a low risk but less profitable food patch, and a high risk but more profitable one.

Birds were observed with binoculars through the windows of an observation hut approximately 10 m from each of the four aviaries. The four replicate aviaries were positioned so that there was a distance of approximately 22 m of lawn between adjacent aviaries that were visible to one another. Thus birds were not likely to be affected by the behaviour of birds in adjacent aviaries or gain a sense of cover from structures outside the aviary.

After an initial acclimatisation period, females were observed daily for two weeks prior to the provisioning of nest boxes, four of which were placed on the solid back wall amongst the cover. Daily observations continued until one week after each female had laid her first egg, which included the period in which all eggs were laid along with at least two days after laying. For analysis, a consecutive three week period starting two weeks prior to the laying of the first egg and ending one week after the first egg was laid is considered.

Feeders were replenished at 0900 hours, and the behaviour of the birds was subsequently observed during this initial feeding bout for three hours. The behaviour of the focal female zebra finches were recorded using a combination of continuous recording and instantaneous sampling (Martin & Bateson 1992). The three hour period of observation was divided into five minute blocks. During the first two minutes of each block, instantaneous sampling was used to record data from each of the four aviaries in turn, namely the zone in which each female zebra finch was in, and with whom and what she was doing. This enabled data to be recorded concerning the extent to which she flocked and fed

with the bengalese finches, with the male zebra finch, the extent to which she stayed in cover, the amount of time spent feeding, and the percentage of feeding time in the high risk patches. In the remaining three minutes of each block, using continuous recording, data concerning the nature and number of flights made by the focal female from one of the four aviaries was collected. Data were collected from each of the four aviaries in turn, such that three minutes of continuous sampling were recorded for each focal female once every 20 minutes. This experiment was repeated twice, thus providing data from eight pairs of breeding zebra finches.

Repeated measures ANOVAs were performed using females (n=8) as subjects and time as the within subject effect. Time was divided into three, week-long periods: two periods in the two weeks prior to laying and one in the week beginning on the day the first egg was laid. A mean value for the parameters in question was calculated for each female for each of the weeks. All data in the form of a percentage were arc-sign transformed before analyses were carried out. In all repeated measures ANOVAs multivariate tests of significance were used whenever the Mauchly's test for sphericity indicated that the univariate tests were not valid.

Results

All eight females completed a clutch during the period of observation (mean $\pm$ SE = 4.125 $\pm$ 0.398). Significant changes were seen to occur in the associations between the female zebra finches and the other birds in the enclosure with her during the course of the experiment. The mean number of bengalese finches in the same zone as the female zebra finches was significantly effected by time (Multivariate repeated measure ANOVA: $F_{2,14}$=14.138, P=0.005, see figure 3), with females showing an increased tendency to associate with bengalese finches as they came toward laying. The percentage of time spent with the male peaked in the week prior to laying, and declined to its lowest point in the week following the laying of the first egg (Multivariate repeated measure ANOVA: $F_{2,14}$=17.245, P=<0.003, see figure 4).

The nature of the females' feeding behaviour also changed during the course of the experiment. The percentage of time spent feeding declined significantly towards the laying period (Univariate repeated measure ANOVA: $F_{2,14}$=6.08, P=0.013, see figure 5). The percentage of feeds made by the females with the male zebra finches also declined markedly at the start of laying (Multivariate repeated measure ANOVA: $F_{2,14}$=39.828, P=<0.001, see figure 6), whereas the percentage of high risk feeds made by the female declined significantly through out the observation period to reach a minimum during the laying and immediate post laying period (Multivariate repeated measure ANOVA: $F_{2,14}$=24.704, P=0.001, see figure 6).

The percentage of time spent in cover was significantly effected by time relative to laying (Univariate repeated measure ANOVA: $F_{2,14}$=6.76, P=0.009, see figure 7), with an increase in time spent in cover towards the laying period. Although no significant changes were detected in the rate at which females made flights (Univariate repeated measure ANOVA: $F_{2,14}$=3.45, P=0.061, see figure 8), the number of solo flights made was significantly effected by date relative to laying (Univariate repeated measure ANOVA: $F_{2,14}$=4.01, P=0.042, see figure 9), with a marked increase in the percentage of solo flights made during the laying and post laying period.

Experiment 2

Methods

Single pairs of zebra finches were housed separately in each of the four outdoor aviaries used in experiment 1, again with eight male bengalese finches. In order to test whether the risk taking behaviour of breeding females was affected by the riskiness of the habitat as well as her reproductive cycle, two treatments were utilised, one in which cover was provided, and one where no cover was provided. In the cover treatment, the aviaries (n=2) were provided with an area of cover, approximately 1 m deep along the length of the aviary in the form of camouflage netting and five 1 m long perches suspended from the roof. Food was provided on the ground at two sites along the length of the cage, approximately 1.5 m away from the nearest area of cover (figure 10a).

The remaining two aviaries were subjected to the no cover treatment, where the two feeding sites were placed at the far end of the aviaries, approximately 5 m from the solid sided end wall of the aviary where perches were provided, but no camouflage netting (figure 10b). The feeding sites consisted of a high and a low risk feeding patch as in experiment 1. Each zebra finch pair was allowed to acclimatise to the experimental set up for 14 days before daily observations started. Four nest boxes were placed on the solid back wall of each aviary after two weeks of data had been collected, and data collection continued until each female was observed for eight days after she laid her first egg.

Birds were fed at 0900 hours, and the behaviour of the focal female was then observed for a three hour period using instantaneous focal animal sampling (Martin & Bateson 1992). The three hour observation period was divided into two minute blocks. Every two minutes the location and behaviour of each of the focal female zebra finches was recorded in turn, together with the identity and number of individuals in the same zone as her. The experiment was carried out once, thus the behaviour of two females was observed for each of the two treatments, those with cover and those without cover.

Repeated measures ANOVA were performed comparing daily means for each treatment relative to laying date as the within subject effect, with the provisioning of cover as the between subject effect. All data in the form of a percentage was arc-sign transformed before analyses were carried out.

Results

All females completed a clutch, both females laying in the presence of cover laid five eggs, whilst those laying in the absence of cover both laid four eggs.

The differences in cover between the two treatments and the effect of the laying cycle were both significant in effecting various aspects of the females' feeding behaviour, and the risks she took associated with it. There was a significant effect of date relative to laying upon the percentage of feeds made by females

with the male to which she was paired (Repeated measure ANOVA: effect of day; $f_{2,1}$=5.00, P=<0.001, effect of cover; $f_{1,2}$=7.88, P=0.107, see figure 11) with birds feeding less often with their paired male shortly after the onset of laying. Though there was no significant effect of cover, birds without cover did tend to feed more with their paired male than those with cover. There was a significant affect of cover upon the percentage of feeds made by the female with birds other than the male to which she was paired (Repeated measure ANOVA: effect of day; $f_{2,1}$=1.54, P=0.135, effect of cover; $f_{1,2}$=89.7, P=0.011, see figure 12) with birds breeding in the absence of cover feeding significantly more often with other birds, particularly in the days following the start of laying. The provisioning of cover and time both had a significant effect upon the percentage of high risk feeds made by the female, with birds breeding in the absence of cover feeding far less often in high risk areas, and with birds in both treatments exhibiting declines in high risk feeding towards the onset of laying (Repeated measure ANOVA: effect of day; $f_{2,1}$=3.64, P=<0.001, effect of cover; $f_{1,2}$=432.86, P=0.002, see figure 13). There was a also significant effect of cover and time upon the duration of high risk feeds made by the laying females with birds in both treatments exhibiting declines in high risk feed duration towards the onset of laying (Repeated measure ANOVA: affect of day; $f_{2,1}$=4.82, P=<0.001, affect of cover; $f_{1,2}$=831.97, P=0.001, see figure 14), and with females laying in the absence of cover, feeding for significantly shorter duration in the high risk patches than females laying in the presence of cover.

Discussion

The importance of declines in muscle condition due to laying in affecting alarmed flight performance and subsequently predation risk in birds has already been demonstrated (see Chapter 3), and there is a large body of evidence which suggests that cover is also influential upon predation risk in birds (Lima & Dill 1987b, Bednekoff 1996, Slotow 1996, Beck & Watts 1998). In this series of experiments we have attempted to see whether female zebra finches possess the ability to assess these aspects of predation risk, and subsequently adjust their behaviour in order to influence their risk of being predated.

Work by Jones (1991) on house sparrows (*Passer domesticus*) and by Houston *et al.* (1995a) on zebra finches has shown that declines in muscle volume seen in laying females occur at least three days prior to the laying of the first egg. Since reduced muscle condition is likely to correlate with an increased likelihood of being caught during any single given predatory attack (see Chapter 3), one might expect females to adjust their behaviour at this time in order to minimise this risk. However, at a similar time, one might also expect females to adjust their foraging behaviour in order to increase food intake over this energetically and nutritionally demanding period (Robbins 1981, Walsberg 1983), a behavioural strategy which would conflict with attempts to minimise predation risk (Lima 1987b). However evidence suggests that zebra finches in captivity decrease activity levels and no increase in food intake is seen immediately before and just after the onset of laying (Houston *et al.* 1995c). Although it has been suggested that birds may reduce activity at this time in order to minimise potential damage to developing eggs (Fogden & Fogden 1979), it is suggested here that this is not the case, since during the course of a series of experiments investigating the effects of body mass and egg production upon flight performance, 37 females were caught, handled and flown on a total of 2,505 occasions when gravid, and on no occasion were indications of egg damage such as deformed or damaged eggs being laid or peritonitis seen. It can be assumed therefore, that for much of the daylight hours at least (i.e., the period in which gravid females were flown and handled), zebra finches are resistant to egg damage due to flight. Therefore the reductions in activity seen in laying zebra finches would appear to be an adaptive response to some other variable. Although the reduction in flight activity seen in females relative to laying date in the first experiment was not significant, it is likely that this results from the relatively large standard error of the mean due to the rarity of the behaviour, rather than from there being no real change (see figure 8). However, there were significant changes in other behaviours potentially indicative of attempts to reduce predation risk.

Female zebra finches were shown to associate with bengalese finches more in the week preceding and immediately following the onset of laying (figure 3). This may result from the fact that females were more often in cover at this time (see figure 7) due to the introduction of the nestboxes in the covered part of the aviary, which is where all birds including the bengalese finches spent most of their time when not feeding. However, the increase in time spent in cover, and the increased association with bengalese finches can also be interpreted as an attempt to minimise predation risk during the vulnerable laying period, particularly as the extent to which females associate with bengalese finches appears to increase earlier than the increase in time spent in cover. Since as group size increases, individuals can reduce their own vigilance without necessarily sacrificing predator detection (Lima 1987b), the female may be able to maintain her rate of food intake at a time when it might otherwise have been reduced as a result of the need for increased vigilance by choosing to feed with flocks of bengalese finches. Thus, the reduced time spent in the vicinity of the feeding stations towards the laying period, may not necessarily indicate a reduced food intake, since females may be more efficient feeders at this time.

The females' association with the male (see figures 4, 6, 11) might change for three reasons: 1. The male will preferentially associate with the female when she is most fertile to ensure paternity of offspring (Birkhead *et al.* 1988, Burley *et al.* 1994). 2. The male will spend less time with the female away from the nest during the incubation phase since both sexes share incubation (Birkhead *et al.* 1988). 3. The male and female may associate more when her vulnerability to predation increases, so as to increase their corporate vigilance and thus chances of detecting approaching predators (Hamilton 1971, Pullman 1973, Kenward 1978), and to maintain the females' rate of food intake (Lima 1987b). It is apparent that the associations between the male and female are driven more by reasons 1 and 2 than by 3, since at the time when her predation risk is highest, (i.e. in the week following the start of laying), the male spends the least time with her. The decrease in time spent with the male, including feeding time and the increase in solo flights made by the female in the week following the start of laying (figure 9), is most likely to be due to his attendance and

incubation at the nest and the reduction in the need to guard against extra-pair copulation. The peak in time spent with the male coincides with the time at which she is most fertile, but not necessarily when she is most at risk from predation. In fact an analysis of the daily data for all eight birds in experiment 1 revealed that on the day after the female laid her first egg, and the first day on which the male can be sure that she is fertile, all males associated with their respective female for 100% of the observed time. This coincides with the peak in the rate of extra-pair courtship's performed by males toward females (Birkhead *et al.* 1988)

Figures 4, 6, 9 and 11 demonstrate that over the time period when the females' escape flight capability declines and her degree of association with the male decreases, female zebra finches show a marked shift in food patch preference away from the high risk patches and also reduce their overall time spent feeding (see figures 5, 6, 13, 14). Although it has been shown that zebra finches do not increase their daily intake of food at the time of laying (Houston *et al.* 1995c), our results would appear to indicate that they may in fact reduce their food intake, or at least the time spent feeding (see figure 5). Since egg production is an energetically demanding time for birds (Walsberg 1983) and it has been shown that reproductive success declines in zebra finches as the net rate of energy gain decreases (Lemon & Barth 1992), the reduction in feeding behaviour, and most notably in the percentage of time spent at the most profitable but riskiest food patches (see figures 6, 13 14), must have a strong adaptive significance. It is suggested here that females are actively adjusting their behaviour in order to minimise their predation risk at a time when it may be elevated due to reduced flight performance.

Although results from the second experiment should be treated with caution due to the small sample sizes, similar apparently adaptive changes in behaviour were seen as a result of the onset of laying, namely a reduction in the proportion and duration of feeding bouts at the high risk patches and a tendency toward an increase in feeds with birds other than the paired male as the females began laying. However, in all cases, the females' response

appeared more extreme in the absence of cover than when birds were given access to cover: thus females fed more often with bengalese finches and made fewer high risk feeds which were also of a shorter duration in the absence of cover, than in the presence of cover. The reduction in feeding behaviour with the paired male, is again likely to be a result of shared incubation and a decline in the need to mate-guard. Since there was no variation in clutch sizes between the cover and no cover treatments, and sample sizes were small, statistical tests on clutch size could not be performed. However, it is possible that a reduction in clutch size (from five eggs to four) in the absence of cover may represent a physiological adaptation to minimise any potential increase in predation risk resulting from laying in this relatively more hazardous environment.

The findings from these experiments would appear to confirm the hypothesis that egg production is likely to result in an increase in predation risk in zebra finches, since the changes seen in female behaviour, such as a decrease in time spent in the high risk feeding patches are unlikely to have occurred if this were not the case. However, if this were the case, this would surely increase their predation risk. Perhaps more significantly, these findings demonstrate that female zebra finches clearly posses a behavioural flexibility which allows them to modify their behaviour in such a way as to compensate for the reduced ability to make rapid escape flights during the laying period.

Fig. 1.

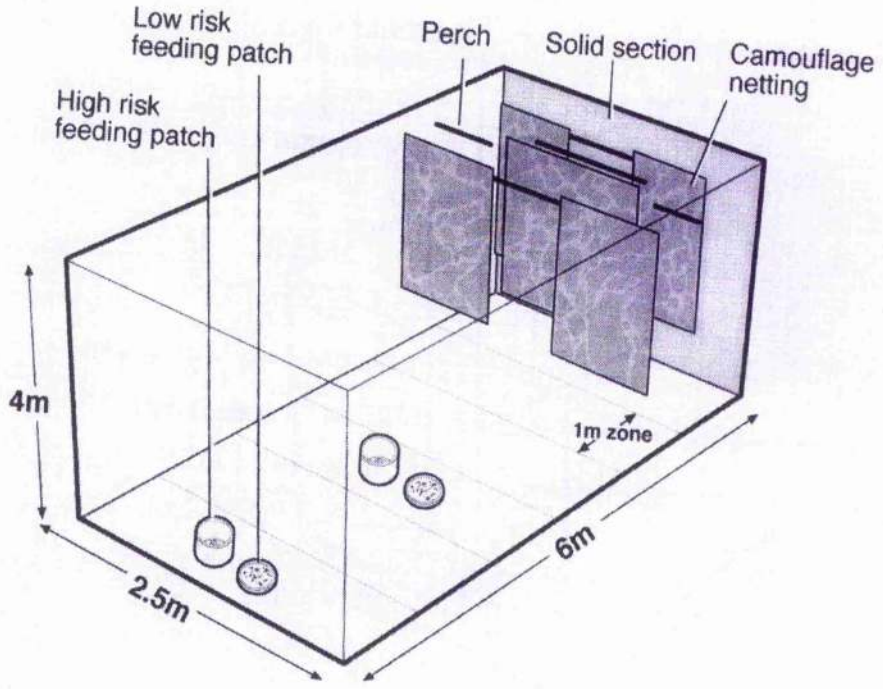


Fig. 1. Schematic representation of the outdoor flight aviaries used in experiment 1.

Fig. 2.

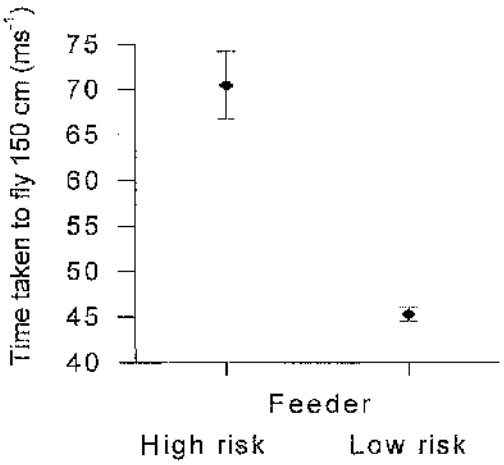


Fig. 3.

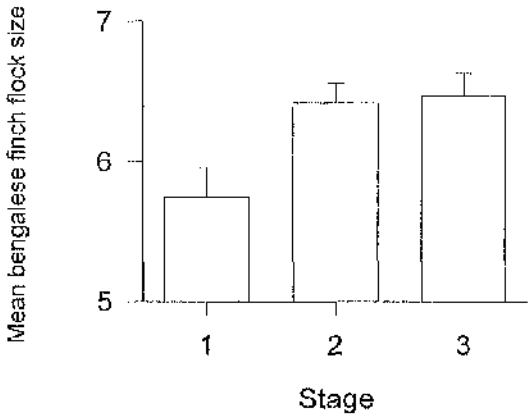


Fig. 2. The effect of feeder type upon the mean (±SE) time taken for female zebra finches to fly 150 cm from the feeder when startled.

Fig. 3. The effect of stage of laying on the weekly mean (±SE) number of bengalese finches in the same zone as the female zebra finch. Stage 1= the week starting two weeks prior to the onset of laying, stage 2 = the week starting one week prior to the onset of laying, stage 3 = the week starting with the onset of laying.

Fig. 4.

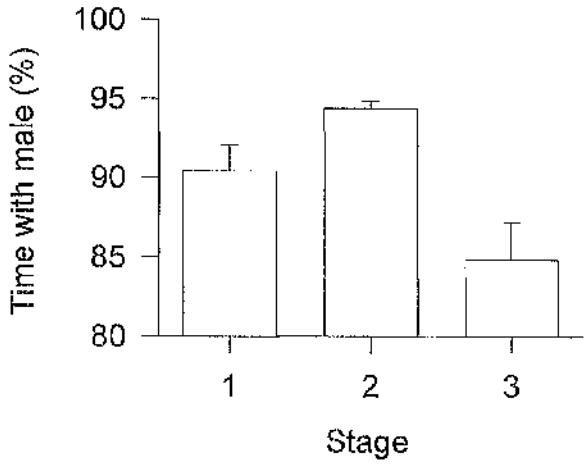


Fig. 5.

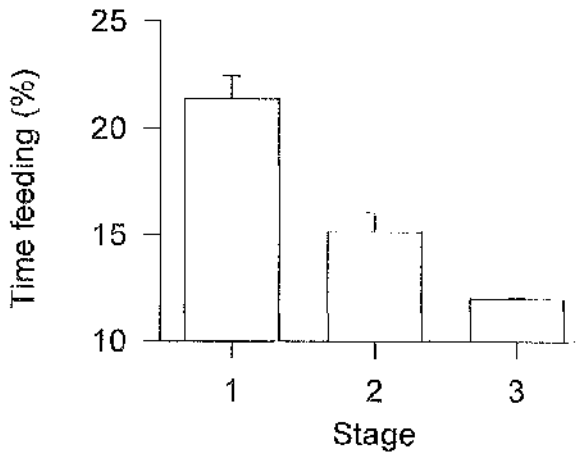


Fig. 4. The effect of stage of laying on the weekly mean (+SE) percentage of time the male zebra finch was in the same zone as the female zebra finch.

Fig. 5. The effect of stage of laying on the weekly mean (+SE) percentage of time spent feeding by the female zebra finch.

Fig. 6.

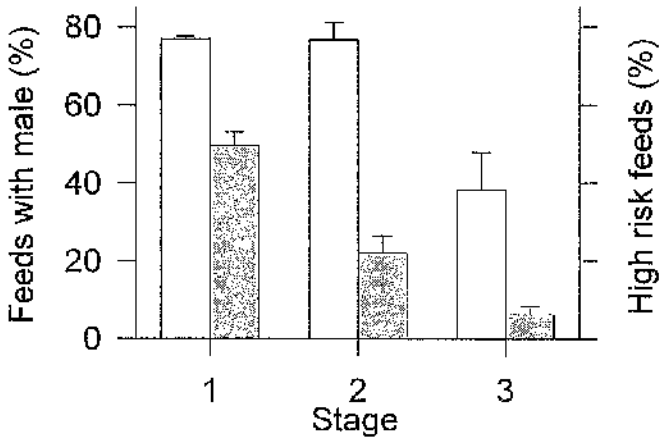


Fig. 7.

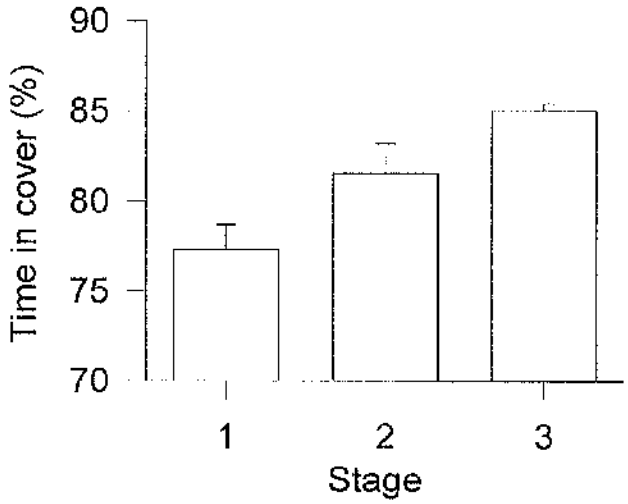


Fig. 6. The effect of stage of laying on the weekly mean (±SE) percentage of feeds made with the male by the female zebra finch (open bars) and the weekly mean (±SE) percentage of high risk feeds made by the female (shaded bars).

Fig. 7. The effect of stage of laying on the weekly mean (±SE) percentage of time spent in cover by the female zebra finch.

Fig. 8.

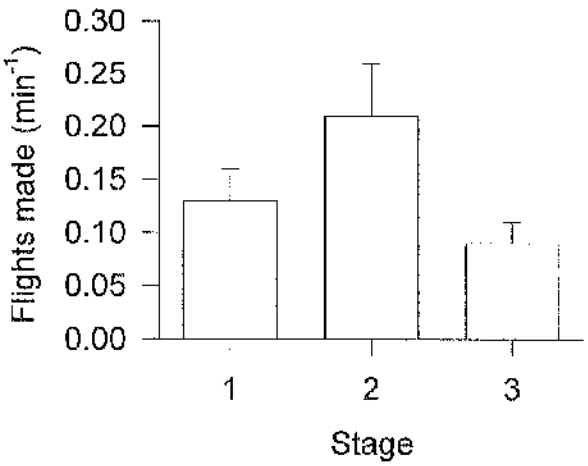


Fig. 9.

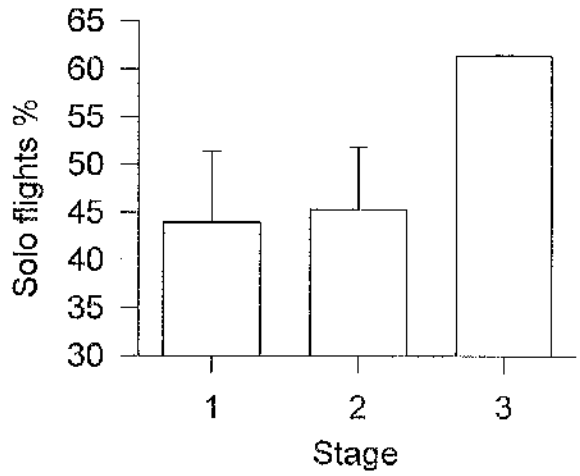


Fig. 8. The effect of stage of laying on the weekly mean (±SE) number of flights made per minute by the female zebra finch.

Fig. 9. The effect of stage of laying on the weekly mean (±SE) percentage of solo flights made by the female zebra finch.

Fig. 10. a.

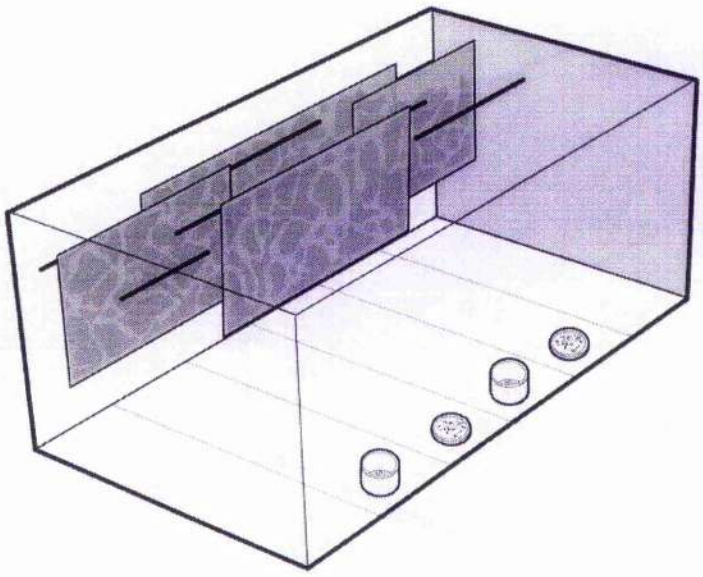


Fig. 10. b.

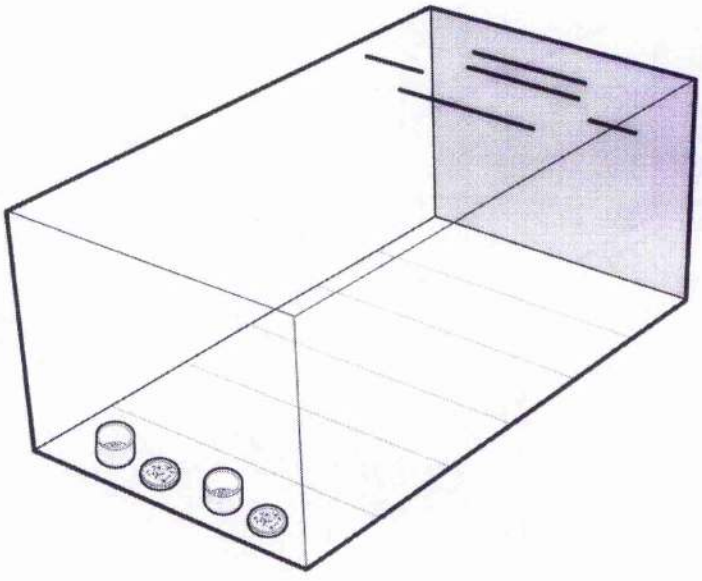


Fig. 10a. Schematic representation of the outdoor flight aviaries used in experiment 2 for the cover treatment. Fig. 10b. Schematic representation of the outdoor flight aviaries used in experiment 2 for the no cover treatment. Refer to Figure 1 for further details.

Fig. 11.

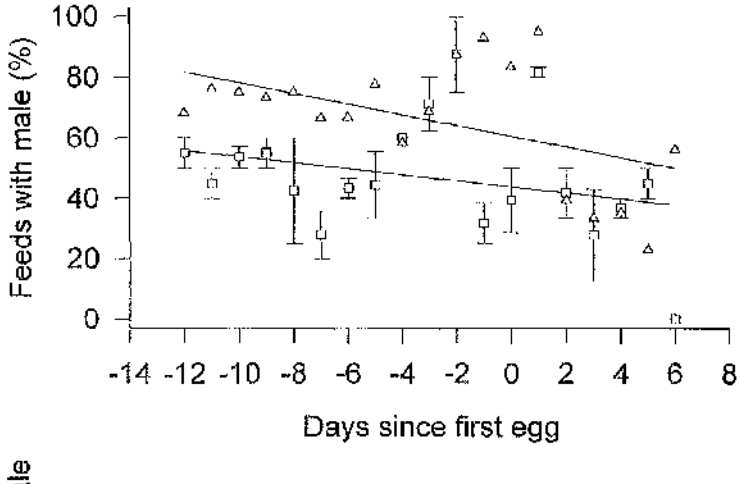


Fig. 12.

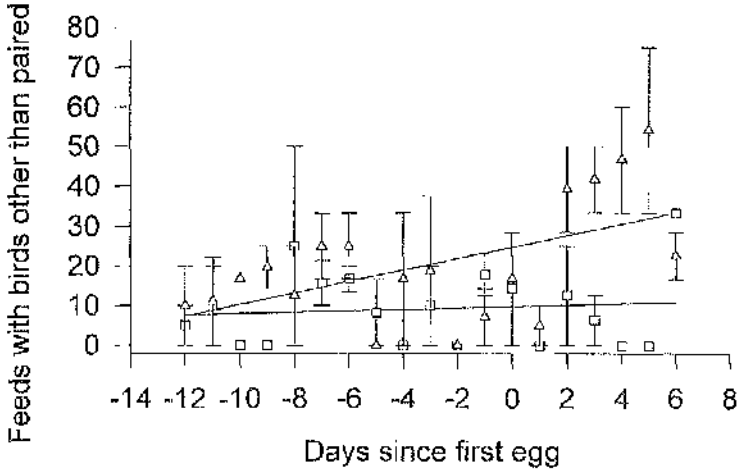


Fig. 11. The effect of date relative to the laying of the first egg (day 0), and the presence or absence of cover upon the mean (±SE) percentage of feeds made with the male by the female zebra finch. No cover treatment = triangles, cover treatment = squares.

Fig. 12. The effect of date relative to laying, and the presence or absence of cover upon the mean (±SE) percentage of feeds made by the female with birds other than the male to which she was paired. No cover treatment = triangles, cover treatment = squares.

Fig. 13.

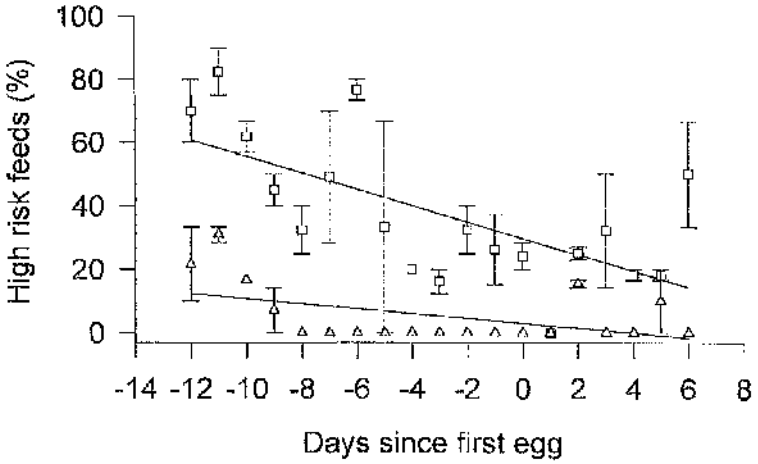


Fig. 14.

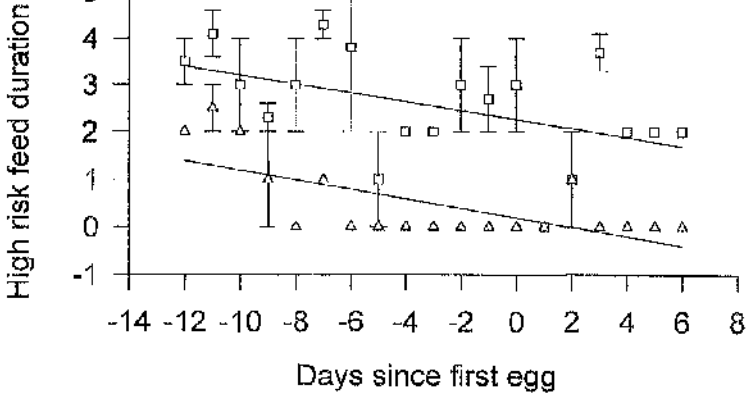


Fig. 13. The effect of date relative to laying, and the presence or absence of cover upon the mean (±SE) percentage of high risk feeds made by the female zebra finch. No cover treatment = triangles, cover treatment = squares.

Fig. 14. The effect of date relative to laying, and the presence or absence of cover upon the mean (±SE) high risk feed duration made by the female zebra finch. No cover treatment = triangles, cover treatment = squares.

Chapter 8

Manipulating the nesting biology of endangered bird species: problems and solutions

Abstract

The removal of eggs from the nests of rare wild bird species is a widely used conservation tool designed to increase reproductive output and subsequently bolster endangered populations. Although it has been used with some success, there are problems associated with this method which may compromise its effectiveness as a conservation tool. Here relevant research which outlines these problems is reviewed and suggestions as to how these problems can be limited are made.

Introduction

Egg removal is a now widely used management tool utilised in order to increase fecundity in endangered bird populations. In this procedure, freshly laid eggs are removed from nests as soon as they are laid, thus encouraging females to lay eggs to replace those which have apparently been lost. Subsequently a larger than normal clutch is laid. Alternatively the entire clutch may be removed at the end of laying to encourage the female to lay a full replacement clutch, a technique known as double clutching (see Haywood 1993ab for details concerning the sensory cues which control this). The removed eggs are typically incubated in controlled conditions, either using a foster species or an incubator. This method has been used on the Mauritius kestrel (*Falco punctatus*) (Jones *et al.* 1994), the Californian condor (*Gymnogyps californianus*) (Toone & Wallace 1994), the black stilt (*Himantropus novaezelandiae*) (Reed *et al.* 1993), the black robin (*Petrocia traversi*) (Merton 1990, Butler & Merton 1992), the echo parakeet (*Psittacula eques echo*) (Jones & Duffy 1993) the takahe (*Porphyrio mantelli*) (Clout & Craig 1995), the kakapo (*Strigops habroptilus*) (Clout & Craig 1995) the osprey (*Pandion haliaetus*) (Poole 1989) the peregrine falcon (*Falco*

perigrinus) (Saar 1988) and the prairie falcon (*Falco mexicanus*) (Cade & Temple 1994) to name but a few.

Egg removal and subsequent artificial rearing, however, has certain limitations. Firstly, removed eggs need to be incubated and chicks reared either by a foster species or by hand in captivity, with birds being released at a later date. Difficulties with cross-fostering to a different species and the subsequent release of captive bred young have been recognised in the past. Chicks from Takahe eggs for example, cross fostered to bantams imprinted on the foster parents (Clout & Craig 1994), and interspecefic mating has been seen to occur in cross fostered raptors and between whooping cranes (*Grus americana*) fostered to sandhill cranes (*Grus canadensis*) (Cade & Temple 1994). Secondly survival of captive bred birds may be low in the wild (Clout & Craig 1994, Cade & Temple 1994). Finally the assumptions which underlies this management tool, which is that increased egg production has no negative consequences on the quality of egg produced or maternal condition is flawed, and it is this area that is considered in detail here.

The assumption that egg production is inexpensive has risen from the apparent ease with which females typically replace lost eggs (Lack 1947), no doubt reinforced by the widespread knowledge that domesticated chickens (*Galus domesticus*) can typically lay 80 eggs in 80 successive days. Recent studies on birds, both in captivity and in the wild, have shown that egg production may in fact be costly (Houston *et al.* 1995a, Ward 1996, Monaghan & Nager 1997, Nager *et al.* in press), and is expensive in terms of energy and nutrient requirements as well as breeding performance and potentially female survival (Chapter 5, Slagsvold & Dale 1996, Monaghan *et al.* 1998). The excessive depletion of protein reserves following clutch manipulations may also result in the female being less able to attract a mate (Monaghan *et al.* 1996). Consequently increased egg production can be potentially costly and needs to be taken into account by conservation biologists when using this technique.

Here experimental evidence is reviewed which suggests that increased egg production negatively affects female body condition with consequences on her own flight performance, the quality and sex ratio of her offspring, and her attractiveness to mates. Although the cited studies come from common bird species, the underlying processes are likely to be the same in endangered species as well. An outline of how these negative effects can be reduced is then made, such that the effectiveness of this useful tool can be maximised.

Female condition

It has been shown that in 21 of the 29 bird species for which female muscle condition has been examined, there is a significant loss of muscle mass from the body during laying (Houston *et al.* 1995a) as protein is transferred from muscle tissue into the formation of eggs (Houston *et al.* 1995c). Much of the musculature concerned is involved in locomotion, both ambulatory and flight (Houston *et al.* 1995c). For example, Houston *et al.* (1995c) found that 14% of lean flight muscle mass, and 15.3% of lean leg muscle mass is lost in the zebra finch (*Taeniopygia guttata*) as a result of laying. Protein from muscle reserves may be used to provide essential amino acids which are limiting in the diet at the time of egg formation (Houston *et al.* 1995c). Egg proteins have an unusual amino acid balance compared to other proteins as they posses high levels of the amino acids cystine and methionine (Houston *et al.* 1995a), and subsequently, it is the quality of proteins in the diet of birds which limits egg production rather than the absolute quantity (Ramsay & Houston 1997). Moreover, there is good evidence that it is the quality of muscle proteins rather than the absolute amount of muscle which influences egg production, and that this muscle quality is influenced by the nutritional balance of the diet in the period prior to breeding (Selman & Houston 1996a).

Such losses in muscle tissue are likely to effect the locomotor capabilities of females, and subsequently the ability of the bird to forage and to escape from predators, since the ability of a bird to get airborne rapidly is the single most important factor determining the chances of survival when under attack from avian or mammalian predators (Rudebeck 1950, Page & Whitacre 1975,

Cresswell 1993, Bednekoff 1996). Work carried out on captive zebra finches has demonstrated that by encouraging females to lay an extra egg (mean clutch ± standard error = 4.9±0.3), female muscle condition declines to such an extent that post laying flight performance at take-off is reduced by 7% compared to when the same females were encouraged to lay a smaller clutch (mean clutch = 3.6±0.2) (Chapter 5). In the time represented by this 7%, a pursuing raptor may be capable of travelling between 70-120 cm further towards the escaping bird, thus severely lessening the chances of survival once an attack has been initiated (Chapter 5). This is particularly relevant to many endangered bird species, since a large proportion have become endangered due to the introduction of alien predators (Clout & Merton 1998, Lovegrove 1996, Imber *et al.* 1994).

The findings outlined in Chapter 6, together with those of Selman & Houston (1996a) clearly demonstrate that the extent to which females rely upon muscle tissue in the formation of eggs is dependent upon the protein quality of their pre-breeding diet. Veasey *et al.* (Chapter 6) encouraged female zebra finches to lay the same sized clutches under different regimes of protein supplementation. When birds produced a clutch on a high quality protein pre-breeding diet, they actually gained muscle condition during the course of laying (+3.45%±2.4), whilst when birds laid a clutch having been given a low quality protein pre-breeding diet they lost muscle condition (-8.37%±3.1). This was reflected in the subsequent changes in flight performance: when given a high quality protein pre-breeding diet, females took less time to complete a flight of a prescribed length at the end of laying (-7.15%±3.1) than when given a low quality protein pre-breeding diet (+3.84%±3.4). Thus simply by providing laying females with a high quality diet, in this case homogenised boiled hens eggs and soya rusk, the effects of reproduction upon muscle wastage and subsequently flight performance can be minimised.

That egg production effects maximal flight performance due to a reduction in muscle condition, means that foraging efficiency may also be effected, particularly in insectivorous and predatory birds which rely upon maximal flight performance to obtain food. Work carried out on lesser black backed

gulls (*Larus fuscus*) (Monaghan *et al.* 1998) and common terns (*Sterna hirundo*) (Heaney & Monaghan 1995) has shown that by encouraging females to lay an extra egg, the capacity of the parents to provision for a control brood is reduced. It should be noted that in both cases, the chick rearing period is several weeks after laying, and so the capacity for females to compensate for the demands of laying an extra egg during the later stages of the same breeding attempt is limited. The costs of egg production for the parent may therefore be long term. McCleery *et al.* (1996) found that a drop in nest predation which resulted in increased clutch sizes in the great tit (*Parus major*), gave rise to increased female mortality after 5 years old, related to the number of successful breeding attempts she had made, though no such relationship was found for males. It was suggested that this may have resulted from increased competition, however such a relationship should then also have occurred in males who would surely be subject to similar levels of competition. Since male great tits also help in chick rearing, one might assume that the increased female mortality is related to the costs associated with egg production. It is possible for example that the increase in clutch size increased the likelihood of females being predated or contracting diseases or parasites due to a reduction in their condition, a factor that became more evident after 5 years old due to physiological deterioration with age exaggerating the costs of reproduction.

Reproductive effort during the chick rearing period (rather than egg laying) has also been shown to reduce antibody responsiveness in birds (Deerenberg *et al.* 1997). Here clutch sizes were manipulated in captive zebra finches and enhanced effort was found to affect the production of antibodies toward sheep red blood cells (Deerenberg *et al.* 1997). This reduction in immunocompetence associated with reproduction may explain the increased parasitism observed in numerous species during the reproductive period (Deerenberg *et al.* 1997). The effort associated with rearing young, independently of laying, has been shown to render birds more vulnerable to parasitic infection (Gustafsson *et al.* 1994, Deerenberg *et al.* 1997, Nordling *et al.* 1998). A reduction in immunocompetence appears to come about as a result of the adaptive reallocation of limited resources during chick rearing (Deerenberg *et al.* 1997).

It is possible therefore, that the allocation of resources to egg production will also compromise immunocompetence. Subsequently, the provisioning of extra resources during the laying and pre-laying period may reduce the extent to which birds become immunosupressed (Baron 1988).

Offspring quality

When lesser black-backed gulls were encouraged to lay additional eggs, the resultant eggs proved to be of poorer quality (Nager *et al.* in press). Although the egg mass of additional eggs did not decline below the mean egg mass of normal last eggs, the proportion of lipids in the egg, the main energy source for the growing bird decreased, whereas the proportion of water increased (Nager *et al.* in press). In a similar experiment on common terns, additional eggs showed a significant decrease in the thickness and relative weight of the eggshell (Heaney *et al.* 1998).

That these changes in egg quality have an effect upon chick survival is shown by the poorer success of additional eggs when fostered to unmanipulated control pairs. In lesser black-backed gulls, additional eggs had a poorer hatching success and successful hatchlings had poorer chances of survival to fledging compared to control eggs (Nager *et al.* in press). A chick hatched from the first egg of an experimentally extended laying sequence had an average survival probability of 90%, whereas this declined to 31% for a chick hatched from the 11th egg. This effect did not result from the poorer condition of the mother, as eggs were transferred before incubation started to control pairs unaffected by increased egg production.

Examples of reduced offspring quality in managed populations may be evident in a number of endangered species. In the Mauritius kestrel for example, females were made to lay up to four clutches. Egg quality however declined in later clutches and no fourth clutch ever included fertile eggs (Jones *et al.* 1994). The Mauritius kestrel was also induced to lay extended clutches by the removal of fresh eggs, however the fertility and hatchability of the additional eggs declined (Jones *et al.* 1994).

It is presently unclear what factors cause the poorer success of additional eggs. It is typically assumed that chicks hatched from larger eggs are more successful (Williams 1994). In the gull study, success of the egg was not only related to its mass but to its position in the laying sequence; additional eggs had a significantly poorer success than normal eggs despite being of a similar size (Nager *et al.* in press). This indicates that changes in egg composition may be responsible for declines in egg quality in additional eggs. Alternatively, the immune system of the laying female may compete for protein resources with the process of egg formation, consequently offspring from additional eggs obtain smaller amounts of maternally transferred antibodies and might thus have a compromised immunocompetence and therefore may have lowered chances of survival.

So far only effects of increased egg production on females and subsequently her eggs has been considered. However, by inducing larger clutches to be laid, gamete production of the male might also need to be increased. For lesser black-backed gulls there is evidence that additional eggs receive less sperm and are more likely to be unfertilised (Nager, unpublished data).

The quality of additional eggs can be improved by supplementary feeding the female both before and during laying of the manipulated clutch. Zebra finches given high protein supplements prior to breeding tend to lay heavier clutches (Selman & Houston 1996a, Monaghan *et al.* 1996) or heavier individual eggs if encouraged to lay similar sized clutches (Chapter 6). Since various parameters of offspring fitness have been shown to relate to egg size (Smith & Bruum 1998, Hill 1993, Williams 1994, Amundsen 1996), the advantages of supplementation for endangered birds prior to laying is evident.

Offspring sex ratio

In sexually dimorphic species, the larger sex is often more susceptible to changes in environmental conditions (Sheldon *et al.* 1998). Insufficient resources in additional eggs might therefore have a larger negative effect on the

bigger sex. In lesser black-backed gulls males grow faster (Griffiths 1992) and are approximately 12% larger than females when adult (Cramp 1983), but are also already larger at hatching (Nager *et al.* 1992). Males hatching from additional, poorer quality eggs, have a higher chick mortality than females hatching from additional eggs (Nager *et al.* 1999). This difference in survival between males and females was independent of the condition of the female after laying since they all had been cross-fostered to, and were raised by, unmanipulated parents. This sex-difference in survival will bias the sex ratio of young. Furthermore, to improve their reproductive success under the given poor condition, female lesser black-backed gulls produced an increasing proportion of daughters with decreasing body condition: among last additional eggs these were 75% females (Nager *et al.* 1999). Such extreme sex ratio biases can have very important negative impacts on the effective population size and thus counteract the effects of increased fecundity. Although there are potential advantages to this in polygamous species such as the kakapo, it is not known which way, if any, a sex ratio bias may go. Since the difference in offspring performance between the sexes depends on egg quality, the effects on offspring sex ratio can be mitigated by supplementary feeding as demonstrated by the lesser black-backed gull study (Nager *et al.* 1999).

General conclusions

There are numerous unwelcome costs associated with clutch manipulation that are likely to jeopardise the survival of parents and offspring, and thus the effectiveness of egg pulling as a conservation tool. However, all of the physiological costs can to some extent be negated by the provisioning of high quality food supplementation which also has the beneficial effects of increasing fecundity (Drent & Daan 1980, Nager *et al.* 1997), and may even increase the likelihood of pairing, since male zebra finches have been shown to selectively pair with females which have been exposed to a high quality diet (Monaghan *et al.* 1996). This may be particularly important for small populations where the choice of mates is limited. It is notable that on Little Barrier Island, translocated kakapo bred only after being provided with food supplementation (Powlesland & Lloyd 1994), seven years after having been transferred there.

However the low hatching success of subsequent eggs, can possibly be attributed to the poor quality of the supplementation provided.

Though there are certainly problems associated with the presentation of food supplements for some wild bird species (Jones & Duffy 1993), persistence is advised since the benefits are likely to be high: food supplementation is likely to affect subsequent parental fecundity and survival, attractiveness to mates and chick survival due its effects upon fledging weight (Kilner 1998). Since all bird species may to differ in their requirements, a blanket recommendation cannot be made. Homogenised hard-boiled hens eggs mixed with a commercial rusk and soya meal mix has proved beneficial in the zebra finch (Selman & Houston 1996a). However, such a high protein diet may prove toxic to some species. It must be emphasised once again that protein quality rather than quantity is critical, subsequently the supplementation of limiting amino acids may prove beneficial. Ramsay & Houston (1998) found that blue tits (*Parus caeruleus*) supplemented with the amino acids cystine, threonine, tryptophan, lysine and methionine laid clutches 18% heavier than control birds. Such supplementation regimes may be applicable to others species, since a study by Murphy (1994), found the amino acid composition of egg proteins to be relatively invariant between species. However this study is based on a limited number of species, and so this assumption should not be taken for granted. Research into the amino acid requirements for egg production of each individual species is therefore recommended.

Chapter 9

General Discussion

This series of investigations was prompted by findings from three main lines of research. Firstly, and most obviously is the accumulated evidence which highlights the importance of flight speed for birds in escaping predatory attacks (Rudebeck 1950, Page & Whitacre 1975, Cresswell 1993, Bednekoff 1996). Secondly is research which has followed on from the early work on declines in pectoral muscle condition of laying red-billed quelea (*Quelea quelea*) (Kendall *et al.* 1973, Jones & Ward 1976): Houston *et al.* (1995c) demonstrated similar losses in muscle condition of laying female zebra finches (*Taeniopygia guttata*), and has successfully attributed this to a diversion of muscle nutrients to egg production. Since pectoral muscles are the primary source of power for flight, a reduction in muscle volume is likely to reduce subsequent flight performance. Marden (1987) has already demonstrated that in flying animals, the ratio of flight muscle mass to body mass positively correlates with maximum lift production, and so, all things being equal, one would expect the loss of muscle tissue in egg formation to negatively effect flight performance and hence elevate predation risk. Finally, following on from the theoretical work of Hartman (1961) and Pennycuick (1969), Metcalfe & Ure (1995) have demonstrated that increased body mass tends to reduce flight velocity in the zebra finch. Since birds may be between 7-50% heavier when gravid in comparison to when not breeding (Perrins & Birkhead 1983, Moreno 1989), reproduction may have an effect upon predation risk through the effect mass has upon flight performance.

It is widely accepted that reproduction is costly and may diminish parental survival (see Stearns 1992). In concordance with this, it can be seen that egg production may potentially affect flight performance via the reduction in female muscle ratio during the course of a breeding attempt due to the combined effects of elevated body mass and decreased muscle condition, which in turn will affect predation risk and thus the probability of survival.

Though many studies have investigated reproductive costs, relatively few have investigated parental predation risk as a cost of reproduction in birds. When predation risk as a cost of reproduction has been investigated, it is typically the predation of the chicks or eggs that is considered, or the predation of the parent whilst incubating, and to a lesser extent whilst attracting mates, copulating and when gravid (Magnhagen 1991). Thus, one of the aims of this study has been to redress this balance, and discover the potential costs of egg production alone in terms of predation risk.

In determining the extent to which egg production affects post-breeding maternal predation risk in the zebra finch, a change in alarmed, i.e. maximal flight performance needed to be demonstrated, since this is a better indicator of predation risk than routine flight performance (Veasey *et al.* 1998). The findings of Chapter 5 perhaps most clearly demonstrate how reproduction affects maximal flight performance. When encouraged to lay a small clutch, the flight performance of females were better at the end of laying than at the beginning, which presumably results from the fact that females appeared not to rely on muscle stores and were considerably lighter (8%) at the end than at the beginning, and were not limited in their movements by the presence of developing eggs within their oviducts. However, when encouraged to lay a larger clutch, females lost greater amounts of muscle condition and were subsequently slower flyers at the end than at the beginning of the clutch, despite being lighter and not having eggs within the oviduct.

Having demonstrated that reproduction does affect flight performance, the cause of this change needed to be determined. As has been said, there were two potential candidates which were under consideration; changes in body mass and changes in muscle volume.

The first experimental chapter (Chapter 2) of this thesis therefore sought to determine the exact nature of the relationship between alarmed flight velocity and body mass. Contrary to the theoretical work of Hartman (1961) and Pennycuick (1969), and the empirical work of Metcalfe & Ure (1995), no

relationship between body mass and flight velocity was found. However, most research into this area had not really considered the potential for a difference between alarmed and routine flight velocity. Six reasons potentially explaining why the relationship was different between the two flight types are described in that chapter. Though these results seem somewhat counterintuitive, a number of other pieces of research have confirmed these apparently anomalous findings, but have failed to see the importance of the difference between alarmed and routine flights. Of eight studies investigating the effect of mass upon flight performance (Witter *et al.* 1994, Lee *et al.* 1996, Metcalfe & Ure 1995, Kullberg *et al.* 1996, Kullberg *et al.* 1998, Kullberg 1998, Veasey *et al.* 1998, Lind *et al.* in press), only two (Metcalfe & Ure 1995, Kullberg *et al.* 1996) found mass to significantly effect flight performance. The Metcalfe & Ure (1995) paper was the only paper to consider routine flight in detail, whilst all others were concerned with alarmed flight velocity. The existence of a weak (r^2=0.16) and yet significant relationship between mass and alarmed velocity found in the blackcap (*Sylvia atricapilla*) by Kullberg *et al.* (1996) can possibly be attributed to the fact that the increase in weight considered was 59%, far higher than in any other study. The fact that post-laying flight performance differed between large and small clutch treatments, despite changes in mass being equivalent between treatments (Chapter 5), further supports the assertion that changes in body mass are not critical in effecting post-laying alarmed flight performance. Having thus ascertained that the changes in body mass likely to occur during the production of eggs in female zebra finches is unlikely to have an effect upon the type of flights made when under attack from predators, I was then free to investigate the other most likely cause of reduced flight performance, namely the changes in muscle condition which occur during laying.

A fairly large body of evidence has already demonstrated that muscle loss during the course of egg production to be fairly widespread amongst birds (see Houston *et al.* 1995a). However, it had been suggested by Jones (1990) that such muscle loss would be unlikely to impair locomotion. The investigation carried out in Chapter 3 is the first time this had been tested, and the results

show this was not the case, with losses in muscle condition indices exceeding 6%, resulting in declines in alarmed flight performance, independently of body mass changes. The results of Chapter 5 leant further weight to these findings, having shown that independently of diet and changes in mass, increased egg production resulted in increased muscle depletion and subsequently reduced alarmed flight performance.

Having successfully demonstrated that increased predation risk might well be a cost of egg production in birds mediated through changes in muscle condition, these costs were investigated further in terms of pre-breeding costs relating to storage of proteins in muscle tissue and post-breeding costs related to clutch size.

Since investment in reproduction will negatively affect investment in somatic tissue, this can lead to decreased survival caused by metabolic drain, or an increased susceptibility to pathogens or predators. Since zebra finches do utilise muscle tissue in egg production (Houston *et al.* 1995bc), one would expect females to lay down storage tissue prior to laying in order for them to be able to make the most of unpredictable breeding opportunities (Zann *et al.* 1995) and to minimise muscle wastage due to laying. Such an investment however, may yield costs in terms of the acquisition of nutrients to be stored in the bird's muscles, and in the carrying of those nutrients once stored. Chapter 4 aimed to investigate whether there was any physical evidence that female zebra finches did store nutrients, and whether this was costly to them in terms of predation risk. Females were shown to have significantly greater (16%) flight muscle ratios than males prior to breeding. This increased flight muscle ratio can be interpreted as either resulting from the storage of nutrients, or the need for females to have more musculature to transport eggs prior to laying. However, the fact that in females, increased muscle size was associated with slower flight times appears to support the storage component hypothesis, since the larger muscle volumes negatively affect flight performance and thus is unlikely to aid in the carrying of eggs. It therefore appears that females do actively store proteins in their muscle tissue, possibly in the form of high

molecular weight proteins (Houston *et al.* 1995a), and that in line with life history theory, this storage is costly to them.

Chapter 5 demonstrates that egg production is costly, and the extent to which it is costly is at least in part determined by clutch size. Thus when larger clutches are laid, greater declines in muscle condition and flight performance are seen, though changes in mass are similar. These findings have a significant bearing upon the concept of optimal clutch sizes. Lack (1947) suggested that avian clutch sizes had been shaped by natural selection whereby the most common clutch size should correspond to the clutch which would yield the most surviving young. However, it has since been demonstrated that avian clutch sizes are typically lower than the number which have been shown to yield the most young in brood manipulation experiments (see Monaghan & Nager 1997). The fact that birds do not lay more eggs than they do, when so many species have demonstrated an ability to rear more young (see Monaghan & Nager 1997) has lead to the incorporation of trade-offs into the equation (Stearns 1992), where the effects of reproduction upon parental survival and future fecundity are considered as well as juvenile survival and fecundity. However, the true costs of reproduction in birds have often not been fully appreciated due to the frequent exclusion of the costs of egg production. The findings of Chapter 5 and Chapter 3 demonstrate that egg production is costly through its effects upon the ability of females to escape from predators, and subsequently, post-breeding female predation risk may be a factor which over evolutionary time, may have contributed to a depression in clutch sizes below the maximum which can be laid and reared. The implications of these findings are wider than simply effecting predation risk. The fact that maximal flight performance is reduced means that it is likely that the ability of birds to forage will also be diminished, particularly for pursuit foragers such as aerial insectivores, falcons and many fish-eating birds. Thus reduced flight performance due to egg production will have important implications upon the survival of both young and parents, and is subsequently likely to have been a strong selective pressure depressing clutch sizes below the 'Lack clutch'.

It was then considered important to investigate how the relationship between changes in flight performance and muscle condition are affected by female condition. Selman & Houston (1996a) demonstrated that birds with access to a high protein supplement prior to laying, exhibited smaller declines in flight muscle condition. From the findings highlighted so far, it would be expected that this would have a bearing on predation risk. The results of Chapter 6 clearly support this hypothesis, as it is demonstrated that pre-breeding diet quality, independently of clutch size, significantly affects flight performance, due to its influence upon the changes in muscle condition during laying. Birds given access to high protein supplementation prior to breeding loose less muscle condition and fly faster than when encouraged to lay the same sized clutches but on a low protein pre-breeding diet. In the absence of the findings of Chapter 4 therefore, it would appear prudent for females to build up protein reserves prior to breeding to minimise predation risk after breeding. However, the benefits of protein storage highlighted in Chapter 6 (i.e. a maintenance of post-breeding flight performance), must be weighed against the costs outlined in Chapter 4 (i.e. a reduction in pre-breeding flight performance). Subsequently, it can be seen that female zebra finches may not only face a trade-off between predation risk and clutch size, but also between pre and post-breeding predation risk.

This investigation has so far considered the fixed costs of reproduction and the potential consequences of these costs in terms of evolution and clutch size. However, as these results demonstrate, predation risk will vary during the course of an animal's life. Since animals have more to accomplish during the course of their life than avoid predators, adaptations to predator evasion should be sensitive to current levels of predation risk (Lima & Dill 1989). Subsequently, although predation risk would appear to increase as a result of egg production, it is likely that birds will attempt to ameliorate this by altering their behaviour. Chapter 7 demonstrated that females did indeed alter their behaviour after laying in such a way as might minimise their risk from predation. Females were shown to associate with larger flocks and take less risks associated with feeding in the days following the onset of laying. These

effects were exaggerated if birds were deprived of cover. These findings suggest that females actively alter their behaviour in such a way as would minimise their susceptibility to predation at a time when it might otherwise be elevated due to laying. Although the relationship between the male and female changed in such a way as might elevate predation risk, as the pair spent less time together and fed less often together during the days after laying, this is interpreted as being a result of the need for males to assist in incubation, and the fact that the male no longer needs to guard against extra-pair copulation.

The final chapter (Chapter 8) applied the findings of these and other related studies to the practice of egg pulling, i.e. the manipulation of the nesting biology of endangered bird species in such a way as to increase reproductive output. The removal of eggs from nests to increase productivity in rare birds may reduce the ability of those birds to evade predators, survive infection, provide for young, and may thus be counterproductive. Moreover, offspring sex ratio and quality may also be affected. These problems may be ameliorated to some extent by provisioning of a high quality supplement, and attempts to exclude predators.

Costs such as those measured in this series of experiments are unlikely to be the only costs associated with egg production. It is likely that egg production will incur various life history trade-offs. This might include a reduction in clutch or egg size. The relatively small size of eggs of aerial foragers is widely explained as constrains of egg mass upon foraging strategies (Lee *et al.* 1996). Conversely the relatively large egg size of the Kiwis (*Apteryx spp.*) can be interpreted as resulting from limited costs of egg transportation since they evolved in the absence of predators. The findings outlined so far, indicate that egg production rather than egg transportation may constrain maximal flight performance and is subsequently relevant to predation risk and potentially foraging efficiency depending upon the species. Subsequently, the relatively large egg size of the Kiwis can be interpreted as resulting from the limited cost of egg production rather than egg transport seeing as they are a ground

dwelling, ground feeding group, not reliant upon pectoral muscle condition to acquire food, and have no real ancestral need to escape predation.

The reversed sexual dimorphism of many raptor species, has in the past been interpreted as being due to reducing the effect of additional wing loading resulting from the carrying of eggs (see Lee *et al.* 1996). However, in light of the findings from these experiments, an alternative explanation is conceivable. It is possible that the increased size of females within this group, may enable them to more adequately cope with pectoral muscle loss during laying than if they were smaller, and subsequently had smaller pectoral muscles from which they could draw upon. To some extent, reversed sexual dimorphism is seen in the zebra finch, with females having significantly larger flight muscle ratios than males. A larger overall size seen in many female raptors, might also be an adaptation which minimises the costs of having large pectoral muscles as described in Chapter 4.

As with many pieces of research, this investigation has opened up as many new questions as it has answered old ones. Future research might focus upon the rate at which females recover flight performance and muscle condition after laying, and an investigation into the potential self-regulation of clutch sizes by females in relation to current levels predation risk. Experiments carried out here have shown that females are sensitive to changes in predation risk whether due to environmental or physical factors, and are capable of adaptively adjusting their behaviour in relation to this. It has also been shown that both diet quality and clutch size play a part in affecting muscle condition, flight performance and subsequently predation risk. It is conceivable therefore, that when at a higher risk of predation, or a higher perceived risk of predation, females will reduce their reproductive output to minimise any increases in predation risk at the end of laying. It might also be the case that females lay larger clutches when given access to high quality diets (Selman & Houston 1996a, Monaghan *et al.* 1996) because of their enhanced ability to maintain muscle condition and subsequently flight performance at the end of laying, rather than because they are physically more capable of laying those eggs.

Research into these areas would add greatly to the understanding of reproductive trade-offs pertaining to predation risk in birds.

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